IAFP 2024 ABSTRACT BOOK

International Association for Food Protection®

Advancing Food Safety Worldwide®

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GENERAL SESSION ABSTRACTS

IVAN PARKIN LECTURE

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Digesting Truth: Navigating Food Safety Education in the Age of Misinformation

In the era of instant information and widespread social media, the landscape of food safety education faces unprecedented challenges. For example, misinformation, or false information shared without harmful intent, and disinformation, deliberately spread to deceive, both erode the foundation of evidence-based decision-making. In the realm of science, where complexity and uncertainty are inherent, these practices exploit gaps in public knowledge and understanding, leading to skewed perceptions of risk, the adoption of pseudoscientific beliefs, and resistance to scientific consensus on critical issues like climate change, vaccination, and public health guidelines.

The rapid proliferation of such information through social media and digital platforms magnifies these challenges, allowing falsehoods to spread at unprecedented speed and scale. Combatting these forces requires concerted efforts not only to improve scientific literacy and critical thinking skills among the general public but also to develop more effective communication strategies that can bridge the gap between complex scientific information and accessible, actionable knowledge for the broader population.

This presentation will commence with an exploration of the current state of food safety misinformation, identifying several pervasive myths and the mechanisms by which they spread across digital platforms. By examining case studies, we will shed light on the impact of these falsehoods on consumer behavior and public health. Central to the discussion will be innovative strategies for food safety professionals to effectively communicate scientific truths. This includes leveraging new technologies and social media platforms to disseminate accurate information, as well as engaging with online communities to foster a culture of critical thinking and informed decision-making. Furthermore, the lecture will emphasize the importance of interdisciplinary collaboration among academics, government, and industry professionals in combating misinformation and disinformation. By uniting experts in food science, psychology, technology, and communication, we can develop more effective methods for educating the public and advocating for evidence-based practices.

The lecture will conclude with a call to action for food safety professionals, educators, and communicators to take a proactive stance against misinformation and disinformation. Through collective efforts, we can enhance the public's ability to "digest truth," ensuring a safer and more informed society on the topic of food safety and science in general.

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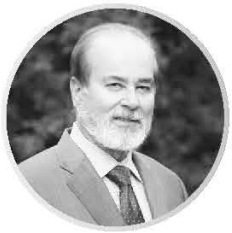

JOHN H. SILLIKER LECTURE

ROBERT BRACKETT

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The Future of Food Safety: Future Shock?

Over the past half century, food safety has made great strides, thanks in large part to technological and analytical advancements. During this same 50+ year timeframe, we have also experienced major and rapid changes in other areas of life. We have experienced major changes in society, including the expansion of social media, communications, entertainment, laws and regulations, business and education, technology, and analytical capability. While one may at first not be able to see the connection between these changes and food safety, they have in fact impacted food safety, both in terms of benefitting but also hindering the advancements in food safety. So, what specifically are these changes that we've seen and how are they related to food – and in particular food safety? Some examples of rapid changes that affect food safety follow.

One of the most impactful changes which we need to consider is social change and, specifically, the role of social media. On one hand, social media has enabled food safety professionals to connect and interact with both existing and new colleagues in ways never before possible. This has allowed individuals to more quickly share and adopt new information. On the other hand, social media has also enabled more rapid dissemination of misinformation and food safety myths. Consequently, there is a need going forward to think more critically about the information to which one is subjected.

Another area in which rapid change is impacting food safety is technology, both digital and analytical. The combination of a faster internet, molecular biochemical methods, and automation has and will continue to enable us to identify foodborne pathogens faster and more accurately than food safety professionals in the 1960s could have possibly imagined. However, it is important not to lose focus on the basics and be forever chasing the "next big thing" in food safety technologies. This will result in students not acquiring essential basic food safety knowledge and food safety veterans perpetually feeling like they are falling behind and can never catch up.

So, that leaves us to ask the question: How do we cope with rapid changes and not leave food safety professionals and consumers behind? Perhaps we need a new paradigm on how to communicate and execute 21st century food safety. If we are to deal with rapid change, particularly as it applies to food safety, we must first recognize that this can and likely will profoundly affect how people view food safety. The new paradigm likewise demands changes in our most fundamental thinking on food safety. We must find a way to adopt and understand the new, cutting-edge technologies while at the same time not forgetting the basics that have been and will continue to be foundational to food safety. This starts with educating future generations of scientists and imparting an appreciation for the underpinnings of applied science, such as microbiology, chemistry, and toxicology. With those basics as a foundation, advanced technologies will become more meaningful and useful.

Symposium Abstracts

S1 Annual Outbreak Symposium

LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
BRIA GRAHAM-GLOVER: *FDA, College Park, MD, USA*
ANGELA FIELDS: *U.S. Food and Drug Administration, College Park, MD, USA*
MATTHEW WISE: *U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA*
MELANIE FIRESTONE: *UMN School of Public Health, Minneapolis, MN, USA*
EWEN TODD: *Ewen Todd Consulting LLC, Okemos, MI, USA*
LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
JOYCE CHENG: *Public Health Agency of Canada, Guelph, ON, Canada,*
AARON BECZKIEWICZ: *USDA-FSIS, Washington, D.C., USA*
ANDREA COTE: *U.S. Department of Agriculture – Food Safety Inspection Service, Atlanta, GA, USA*
MARGARET KIRCHNER: *U.S. Food and Drug Administration, Laurel, MD, USA*
PERRI RUCKART: *CDC, Atlanta, GA, USA*

This symposium covers five different topics surrounding specific outbreak investigations and outbreak investigation mechanics. Additionally, a slot will be held open for a late-breaking outbreak highlighting a recent illness outbreak investigation. Montana state officials and FDA will discuss an investigation into illnesses after consuming morel mushrooms. Speakers will describe the epidemiology of the outbreak, laboratory tests conducted and findings, and challenges in the investigation. An overview of several international outbreaks will be provided by Dr. Ewen Todd. Outbreaks covered will include a variety of pathogen types and food sources. In 2022, there was an outbreak of Salmonella infections linked to cookie dough sold by a pizza restaurant chain. Representatives from CDC, FDA, and industry will discuss the epidemiology, traceback, and public communication challenges surrounding this investigation.

This past year FSIS, CDC and public health partners investigated a salmonellosis outbreak associated with charcuterie meats. This is the second outbreak associated with charcuterie meats in the last three years, and the third outbreak involving RTE, fermented, dried products. The presentation will cover an overview of the recent outbreak, FSIS' response activities, and how these investigations inform research priorities, policy, and investigation procedures at FSIS.

Presenters from the Public Health Agency of Canada and US Centers for Disease Control and Prevention will describe this binational outbreak of nearly 600 *Salmonella* infections. This investigation illustrates the importance of effective international foodborne outbreak collaboration that lead to quick product action. The presentation will also describe how the outbreak affected younger and older populations resulting in adverse health outcomes greater than expected of a *Salmonella* outbreak.

S2 Enhancing Food Safety through Genomic Insights: Advancements in Quantitative Microbial Risk Assessments

BARBARA MASTERS: *Tyson Foods, Washington, D.C., USA*
FRANCISCO ZAGMUTT: *EpiX Analytics, Fort Collins, CO, USA*
JANELL KAUSE: *USDA/FSIS, Manassas, VA, USA*

Traditionally, risk assessments have not taken into account how diversity in pathogen virulence affects the estimation of the true risks associated with specific pathogens. This knowledge gap has serious implications for public health and the food industry, as targets are still largely done based on phenotypes. With advances in genomics, it is now possible to better characterize microbial virulence and its implications for foodborne illnesses.

This symposium will highlight the recent advances of incorporating genomics data on virulence into quantitative microbial risk assessments (QMRAs), a modeling approach that estimates the public health impact of specific microbial hazards and can provide a scientific basis for developing and implementing effective risk mitigation strategies for foodborne pathogens in foods. The integration of genomics into QMRAs not only holds the promise of enhancing food safety but also has far-reaching implications for regulatory agencies, food manufacturers, and consumers. It facilitates evidence-based regulations that target high-risk pathogens, such as *Salmonella*, more precisely and helps food manufacturers implement targeted interventions to reduce contamination risks. This transformative approach enables more informed decisions, tailored risk mitigation strategies, and effective allocation of resources.

In this symposium, experts from various fields will discuss regulatory perspectives, risk modeling approaches, and practical applications for ensuring food safety. Through engaging presentations and discussion, attendees will gain an understanding of how genomics data can revolutionize our approach to microbial risk assessment, ushering in a new era of safer food production and protection of public health.

S3 Follow Your Bacteria; A Data-Based Systems Approach for Safe Meat

MINDY BRASHEARS: *International Center for Food Industry Excellence, Texas Tech University, Lubbock, TX, USA*
SARA GRAGG: *Kansas State University, Manhattan, KS, USA*
JOHN SCHMIDT: *U.S. Meat Animal Research Center, USDA ARS, Clay Center, NE, USA*
JOYJIT SAHA: *Kerry, Beloit, WI, USA*

The constant growth in meat production is accompanied by the necessity for improved safety of the final product. During harvesting, processing, and handling operations, food may become contaminated with a wide range of microorganisms. Tools like biomapping could help to understand where and what extent microorganisms are present in the processing line by establishing a quantitative baseline of indicator organisms. Quantification can benefit the meat industry in several ways: from live production (predicting pathogen load, adjusting interventions) to processing (assessing intervention efficacy and formulations) and deciding on the final product usage. One of the most beneficial usages of this data rich approach would be to support the Statistical Process Control (SPC) at plant level and Quantitative Microbial Risk Assessment (QMRA) framework at region level to help the scientific community and food safety authorities to identify specific monitoring and research needs.

Symposium attendees will learn about: (1) Current situation of the beef and pork industry and the importance of biomapping to comprehend changes in microbial load throughout the process. (2) Prevalence of Salmonella in tissues and samples at the abattoir. (3) How the biomapping would help increase the prediction efficacy of SPC and QMRA models to have significant impact on the outcomes and policy decisions. (4) Case study using these tools effectively would help explain the risk at consumer level understanding the need of biomapping in different areas of food sector.

This session will empower stakeholders in meat, antimicrobials and data science industry with the knowledge and strategies needed to navigate the evolving use of data science along with microbiome technology and the importance of biomapping bacteria to foster safer, healthier choices for meat industry practices and intervention technologies.

S4 Safer with Pressure: State of the High-Pressure Processing Industry and Emerging Applications

MARY-GRACE DANAO: *Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA*
BOBBY HARRIS: *Instinct, St. Louis, MO, USA*
SAMPATHKUMAR BALAMURUGAN: *Agriculture and Agri-Food Canada, Guelph, ON, Canada, Canada*

High pressure processing (HPP) is fully recognized by academics, food manufacturers, and global regulatory bodies as a nonthermal pasteurization technology that can be used to inactivate foodborne pathogens, lower concentrations of salts and preservatives, and slow the growth of spoilage microorganisms. In the last 20 years, HPP has been successfully used to pasteurize a wide range of products ranging from juices and beverages, processed meats, dips and sauces, seafood, baby food, ready-to-eat meals, and pet foods. According to Fortune Business Insights, the HPP food market in the United States is currently valued at $3.23 billion and is growing annually at a rate of 8.5%. This growth is fueled by consumer demand for minimally processed foods that are safe and have high nutritional value. Despite its increasing popularity with food manufacturers, many consumers remain unaware of HPP technology and knowledge gaps persist about its efficacy against pathogens, especially in emerging applications. For example, in raw pet food diets and plant-based meat alternatives, HPP efficacy is highly dependent on product formulation.

In this symposium, industry leaders will present (a) an overview of the current state of the industry and applications worldwide; (b) latest innovations on equipment and process automation; (c) challenges in emerging applications in manufacturing pet foods and plant-based meat alternatives; (d) existing knowledge gaps and regulatory hurdles; and (e) efforts by the Cold Pressure Council to lead, facilitate and promote industry standardization, user education, and consumer awareness of HPP.

Since the last time IAFP had an HPP-focused symposium in 2014 (10 years ago!), several advances in HPP technology and applications have occurred in industry. In the last five years, the number of HPP-related research presentations at IAFP has decreased steadily but more and more students from outside the USA (e.g., Australia, Canada, France, Singapore and Taiwan) are attending and presenting their HPP research as posters at IAFP.

S5 Bridging Data Gaps in Microbial Pathogens Along the Aquaculture Value Chain for Fish in Informal Markets: Advancing Science-Based Analysis for Enhanced Food Safety in Low- and Middle-Income Countries

MOHAMMAD ISLAM: *Washington State University, Pullman, WA, USA*
SELIM ALARAPE: *University of Ibadan, Ibadan, Nigeria, Nigeria*
ELISABETTA LAMBERTINI: *Global Alliance for Improved Nutrition (GAIN), Washington, D.C., USA*
CLARE NARROD: *JIFSAN; U of Maryland, College Park, MD, USA*

The goal of this symposium is to share the data collection efforts of the studies supported by Fish Innovation Laboratory (FIL) of the Feed the Future program of the USAID that focused on transmission of microbial pathogens throughout the aquaculture supply chains destined for informal markets. It will discuss the existing data, identify gaps, and potential ways to minimize the gaps so that data can be used to inform future food safety policy efforts aimed at improving consumer health in low- and middle-income countries (LMICs).

Increasing fish production through aquaculture addresses the growing demand for high-quality protein. As the demand for aquaculture products continues to rise, aquaculture systems worldwide are adopting intensified cultivation methods heavily reliant on formulated feeds and the application of various agrochemicals, including antibiotics. The aquaculture industry in Asia and Africa has witnessed substantial progress over the last decade. Despite the remarkable growth, there has been limited emphasis on improving fish safety in terms of contamination by microbial pathogens, including multi-drug resistant organisms. This concern is particularly significant for fish intended for domestic markets.

Existing data on fish contamination throughout the supply chain in LMICs is limited or is fragmented to fully inform food safety risk assessment. The FIL supported several projects in Asia and Africa that aimed at improving fish production as well as safety. Specifically, projects investigated the level of fish contamination with pathogens across the supply chain. This data could play a pivotal role in utilizing risk-based tools to inform policy action to reduce such risk. Having more comprehensive data collection in the future would help policy makers make better informed food safety decisions aimed at evaluating the overall effectiveness of potential risk reduction strategies.

This symposium aims to bring together a pool of investigators who conducted projects under FIL as well as experts in risk analysis to support risk management questions aimed at reducing the burden of pathogens in fish supply chain.

S6 Food Safety of Cheese Brines: Management and Prevention Strategies

CARRIE JONES: *Prairie Farms, Monona, IA, USA*
KATHLEEN GLASS: *Food Research Institute, University of Wisconsin, Madison, WI, USA*
JULIE AUDY: *Agropur Cooperative, Beloeil, QC, Canada, Canada*

Brine systems of many sizes and configurations are used to manufacture a variety of cheese types. Because brines are in direct contact with products for extended periods of time, they are considered to be product contact surfaces with the opportunity to cross contaminate if a pathogen gets into them. During this session we will review the operation and risks associated with brines looking at large continuous systems all the way down to the small static brines used in many artisan operations. We'll review recent research into mitigation and industry experts will share best practices to help ensure the safety of brining systems.

We will touch on best practices for cleaning, sanitation, and monitoring a system as well as the latest research and intervention technologies being used.

S7 Agricultural Water Treatment: Deploying Conventional and Emerging Solutions to Improve Water Quality for Fresh Produce

CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
FAITH CRITZER: *University of Georgia, Athens, GA, USA*
JAY SUGHROUE: *BioSafe Systems, La Quinta, CA, USA*

Numerous survey studies have recovered foodborne bacterial pathogens (*Salmonella* spp., Shiga-toxigenic *E. coli*, and *Listeria monocytogenes*) in agricultural waters across the United States. Agricultural water is a key potential pathway for the pre-harvest contamination of fresh produce.

Effective water treatments are critical to reducing pathogen levels and preventing the contamination of fresh produce in the pre-harvest environment. Interventions to reduce pathogen burden and prevent pre-harvest contamination can be straightforward and require fewer inputs, or more complicated, requiring more inputs. Several conventional (e.g., chemical sanitizers) and emerging (e.g., UV, ozone) commercial solutions are currently available for treating irrigation water to reduce foodborne pathogen levels. Implementing the appropriate solution to improve water quality may not always require a complicated approach and will depend on the inherent water quality. Conventional solutions may be appropriate under certain conditions to provide appropriate quality for irrigation, whereas innovative solutions may be needed, sometimes in combination with conventional solutions, to improve the microbial quality of irrigation water. Improving water quality in the most efficient manner has become increasingly important as surface water becomes a more critical resource in all agricultural operations.

The goal of this symposium is to present conventional and emerging agricultural water treatment solutions, highlight developing future treatment technologies, discuss treatment applications and how well these solutions will meet the industry's evolving needs. The presentations will be beneficial to industry, academic, and government professionals concerned with pre-harvest water quality and treatment applications. Symposium attendees will leave with a better understanding of water treatment options and the future needs of the produce industry as it strives to improve irrigation water quality in an era of reduced water availability.

S8 Learnings and Products of Public and Private Organizations Developing and Implementing Food Safety Foresight Systems and Approaches

MICHAEL FERRARI: *Climate Alpha, New York, NY, USA*
JULIE PIERCE: *U.K. Food Standards Agency, Bristol, UK*
JOHN DONAGHY: *Nestec Ltd., Vevey, Switzerland*

Different Foresight systems and approaches are used to collect intelligence on future developments relevant for food safety and to identify potential food safety risks. The outputs provide decision-makers with an early opportunity to act on emerging foodborne hazards, risks or opportunities. Foresight is key to keeping food safe because it helps to identify known (re-emerging) or new (emerging) hazards/risks and their potential food safety impact. Foresight techniques, such as horizon or environmental scanning and emerging risk identification contribute to early warning and emergency prevention. They are based on scientific research and practical application and complement existing food safety programs of public or private organizations. They are critical to continuously keeping food safe but often they are reactionary. Also, their capacity to anticipate future events is rather limited when they are largely based on surveillance for known hazards.

Some organizations are new on their journey developing and implementing tailored Foresight approaches and techniques. For others, Foresight has already evolved over time. The rise of video conferencing has strengthened human networks, improving information sharing. Internet- or computer-based systems have been developed for information management and decision-support. Artificial intelligence methods have matured into tools that are increasingly being applied for early food safety risk detection. (Re-)Emerging risk signals can now be deduced from big data sets, including those filled with bias and inaccuracies like social media platforms. Through this symposium, three speakers will share their experience of the evolution and will illustrate Foresight applications to collect data and intelligence, assess risks associated with potential hazards and identify those relevant in the context of their organization.

The session is designed for food safety professionals from academia, industry, consumer organizations and government interested in learning about Foresight approaches and systems tailored to different stakeholder groups or jurisdictions and geographies.

S9 Cultivating a Culture of Food Safety: Key Learnings Toward Food Safety Improvement

VANESSA COFFMAN: *Stop Foodborne Illness, Chicago, IL, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland*
JEFF MILLER: *Mars, California, CA, USA*

Food safety culture has evolved as a concept and various methods and tools have been developed to measure food safety culture in food businesses in efforts to ensure food safety. Regulators have also incorporated food safety culture in their existing formal requirements to ensure food businesses meet their obligations to assure food safety. While the impact of a strong, mature food safety culture on food safety performance is recognized, food safety culture improvement strategies are still quite limited and have not yet been validated in food businesses. This leaves a gap between assessment and driving efforts toward food safety improvement, which includes maturing the food safety culture of food businesses.

To begin filling this gap the session will highlight the importance of using maturity models to position the company's food safety culture and elucidate key learnings and best practices from practitioners' viewpoints to improve food safety culture in the food industry. Use of maturity models helps food business to evaluate their current state of food safety culture, to develop improvement plans against a scale of maturity and enables organizations to assess what is required to reach the desired level of maturity. It can also help in understanding the performance of industry peers and how this compares to its own. Key learnings from interviews with practitioners from five companies actively working towards food safety culture improvement will be elucidated. These key learnings were mapped to the Global Food Safety Initiative (GFSI) dimensions – Values and Mission, People, Consistency, Adaptability, and Hazards and Risk Awareness as these dimensions distinguish specific behaviors that support food safety improvements and provide a roadmap for improvement. Speakers will share the food safety culture maturity model and its relevance, results from five case studies and the food safety culture improvement journey of one of the food businesses

S10 Beyond Pathogens: "GRAS" Microbes as Silent Carriers of Antimicrobial Resistance (AMR) Genes: Posing a Challenge to Food Safety

NEETU TANEJA: *National Institute of Food Technology Entrepreneurship and Management, Kundli, India*
CHERYL ARMSTRONG: *USDA, Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA, USA*
SADHANA RAVISHANKAR: *University of Arizona, Tucson, AZ, USA*

While extensive research has documented the role of pathogenic bacteria in the transmission of antimicrobial resistance (AMR), a growing body of evidence now suggests that "Generally Recognized as Safe" ("GRAS") microorganisms may play a pivotal, yet less understood, role in disseminating AMR genes throughout the food chain. Therefore, AMR stands as an urgent and paramount global health concern, casting profound shadows over both food safety and public health. The World Health Organization (WHO) has issued a grave warning, projecting that by the year 2050, drug-resistant infections could precipitate the next pandemic, potentially claiming over 10 million lives annually. This projection would surpass cancer, previously a leading cause of death, in terms of its devastating impact. The ramifications of AMR are not limited to health alone; they also cast a substantial economic burden. Projections estimate that by 2050, AMR could inflict costs of up to $100 trillion on the global economy, resulting from diminished productivity and mounting healthcare expenditures. The objectives of this submission are to (1) foster a comprehensive understanding of the

mechanisms through which "GRAS" microorganisms acquire, carry, and transfer AMR genes, potentially compromising the safety of our food supply as well as address the food industry concerns; (2) discuss the challenges and gaps in current regulatory frameworks related to the surveillance and management of AMR in "GRAS" microorganisms for ensuring food safety; (3) explore innovative strategies and interventions to mitigate the transmission of AMR through "GRAS" microorganisms; and identify areas for future research and collaboration to address this emerging threat to food safety. Our goal is to engage in critical discussions, exchange key research findings, case studies, and regulatory perspectives; and collectively address the complexities of the emerging threat posed by AMR transmission through "GRAS" microorganisms. This symposium will provide a unique platform for researchers, policymakers, industry professionals, and other stakeholders to share insights, exchange ideas, and chart a course towards enhanced food safety and the responsible use of "GRAS" microorganisms in food production.

S11 New Insights into Sampling and Testing Ready-to-Eat Foods: Lot-by-Lot vs. across Food Supply, Practical Considerations, and Risk Assessment

MARCEL ZWIETERING: *Wageningen University & Research, Wageningen, The Netherlands*
ALVIN LEE: *Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA*
JANE VAN DOREN: *U.S. Food and Drug Administration - CFSAN, College Park, MD, USA*
RÉGIS POUILLOT: *U.S. Food and Drug Administration - CFSAN, College Park, MD, USA*
YUHUAN CHEN: *U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, MD, USA*

What's new in sampling and testing ready-to-eat (RTE) finished products and ingredients? It is well-recognized that sampling and testing for foodborne pathogens serves as verification of preventive controls, rather than a control measure *per se*. There are renewed interests in revisiting the basics of sampling and testing products, as well as new insights and a better understanding of the performance of sampling strategies in the context of risk assessment to inform food safety decisions. How can we tackle challenges in collecting data on pathogen prevalence and levels necessary for setting performance objectives and robust risk assessment? How best to take samples from a product lot and composite samples for method validation? What risk modeling tools are available to integrate the performance of sampling plans on individual lots into a risk assessment to estimate the extent to which industry-wide sampling and testing the food supply – along with subsequent removal of contaminated product from consumption – could reduce the risk of illness associated with pathogens such as *Listeria monocytogenes* and *Cronobacter* spp. in RTE foods? Given what's known, and the uncertainty remaining, what can industry take into consideration – including "safe harbor" protocols – when choosing a sampling plan and using testing as verification of preventive controls, for ingredients from suppliers and for finished products from manufacturing of RTE foods? This proposed symposium brings together three speakers to share their research findings and current approaches to addressing practical questions on sampling and testing from the laboratory, risk assessment, and application perspectives.

S12 Global Recommendations on Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables from the Joint FAO/WHO Expert Meeting

ELIZABETH BIHN: *Cornell University, Ithaca, NY, USA*
TONG-JEN FU: *U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL, USA*
ANA ALLENDE: *CEBAS-CSIC, Murcia, Spain*

Fresh fruits and vegetables are an important part of a healthy diet and are protective against many chronic health conditions. Yet, fresh fruits and vegetables continue to be implicated in food safety incidents involving microbiological hazards around the globe. Fresh produce contaminated with foodborne pathogens (e.g., bacteria, viruses, protozoa, helminths) have resulted in numerous outbreaks of foodborne illnesses and trade disruptions.

The Codex Alimentarius Commission (CAC) initially developed the "Code of Hygienic Practice for Fresh Fruits and Vegetables" in 2003 then later revised it in 2010 following a Joint FAO/WHO Meeting on Microbiological Risk Assessment (JEMRA), to address microbiological hazards associated with leafy vegetables and herbs. In addition, several commodity specific annexes were added to the code of practice in later years. To update and expand the information available in the previous JEMRA reports, a series of expert meetings on preventing and controlling microbiological hazards in fresh fruits and vegetables was held to gather recent data, evidence, and scientific opinions on the topic. Over the period from 2021–2022, an expert panel convened to develop recommendations for consideration by Codex on the general principles on prevention and control of microbiological hazards in fresh fruits and vegetables; specific recommendations of control measurements of microbiological hazards in sprouts, leafy vegetable and herbs, berries and tropical fruits, melon and tree fruits, and seeded and root vegetables.

This session will summarize the science-based recommendations along with comments on the timeline for CAC adoption of these recommendations and their impact on global regulatory considerations and harmonization.

S13 Back to Basics: Essential Elements of an Allergen Control Program

KEVIN BOYD: *The Hershey Company, Hershey, PA, USA*
NATHAN MIRDAMADI: *Commercial Food Sanitation, Joplin, MO, USA*
MONICA KHOURY: *Nestlé USA, Solon, OH, USA*

The goals of an effective Allergen Control Program are the prevention of allergic reactions among consumers and product recalls due to unintended allergen presence. In this symposium we are focused on 6 essential elements of an allergen control program.

Hazard Identification/Supply Chain Controls

- Hazard identification is the first step in development of an effective Allergen Control Program
- ACPs are dependent on supplier awareness and capabilities
- Visual inspection of ingredients, auditing of suppliers, and analysis for unintended allergen presence in ingredients are key aspects of supply chain allergen management

Allergen Label Controls

- A common cause of allergen labeling recalls continues to be using the use of incorrect packaging
- The label is the primary point of communication with the consumer, and label verification and controls are critical to put the right product in the right package and to have the right information on that package
- In addition to controls, there are additional strategies that can be utilized to help differentiate products with different allergen profiles both for food-allergic consumers and for production employees

Cleaning Methods (Wet vs. Dry) for Allergen Management

- Introduce both wet and dry-cleaning techniques for allergen management.
- Review the cleaning methodology and risks of each.

• Evaluation of the advantages and disadvantages to consider when determining cleaning methodology.

Allergen Cleaning Validations

• Selecting an appropriate target is essential to a robust allergen cleaning validation

• Success criteria of the cleaning validation will depend on product and target allergen

• Equipment design and procedure are equally important parts of the validation process

S14 Advancements in Sample Preparation for Enteric Virus Detection from Diverse Matrices

DAN LI: *National University of Singapore, Singapore*
MALAK ESSEILI: *University of Georgia, Center For Food Safety, Griffin, GA, USA*
WALTER RANDAZZO: *Tenured Researcher at Institute of Agrochemistry and Food Technology (IATA), Valencia, Spain*
CHARLES GERBA: *University of Arizona, Tucson, AZ, USA*

The upstream sample preparation steps have always been recognized as the bottleneck in applying molecular technologies in enteric virus detection from complex matrices such as food and water. Ideally, methods for pre-analytical sample preparation of complex matrices should accomplish one or all of the following functions: separate target analysts from the matrix, increase target concentration, purify the viruses from extraneous material and non-target biota, achieve volume reduction in bulk samples, produce a homogeneous sample, and exclude inhibitory substances. Unfortunately, matrices such as foods are messy and nearly unlimited in variety, so a "catch-all" preparative method is hard to develop. The currently available standard methods (e.g., ISO 15216, BAM 26) outline the sample preparation steps from a number of relevant matrices including shellfish, soft fruits, leafy and stem/bulb vegetables, environmental surfaces, bottled water, etc. However, there are still many other matrices that have also been reported to be associated with enteric virus transmission in need of further investigation, such as hepatitis E virus from meat products.

In addition, it is difficult to determine the virus infectivity by applying molecular methods for virus detection, potentially causing false-positive detection results and thus unnecessary food waste and economic loss. In recent years, exciting progress on human norovirus, one of the most prevalent enteric viruses, has been achieved with the use of human intestinal enteroids (HIEs) and zebrafish larvae/embryos for determining virus infectivity. Therefore, it is time to revisit the sample preparation procedures in order to adapt to the newly developed infectivity-based assays. Specifically, one must ensure that the sample preparation steps would maintain the virus infectivity, which was not a key consideration for the current standard methods based on molecular detection.

In this symposium, we will discuss the recent progress in sample preparation for foodborne virus detection from various matrices, addressing the new challenges and opportunities.

S15 Generating Practical Data Insights into Foodborne Illness and Disease Exposure Disparities Using Epidemiological and Related Data

ALLISON HOWELL: *The Ohio State University, Columbus, OH, USA*
DANIEL WELLER: *CDC, Atlanta, GA, USA*
LAUREN GRANT: *University of Guelph, Guelph, ON, Canada, Canada*

Recent studies suggest that different persons and communities are differentially disadvantaged toward foodborne illness. However, research into these disparities is limited, and the inequities driving these disparities are understudied and poorly understood. Thus, understanding and addressing disparities in foodborne illness and exposure is both a critical and emerging field. Since food safety equity is an understudied area, there have been limited talks at prior IAFP meetings focused on this field. This symposium seeks to highlight the current state of knowledge on disparities in foodborne illness and exposure while identifying key knowledge gaps and demonstrating how practical insights can be generated using observational data. The symposium will focus on studies that identify and characterize these disparities, and stimulate much-needed discussion within IAFP. Additionally, these talks will highlight how emerging analytical approaches, such as counterfactual probability machines, can be used to disentangle the complicated landscape of illness and exposure disparities while highlighting novel food safety and social determinant of health data sources, such as retail food safety inspection data.

S16 Rapid *Listeria* Detection in Post Lethality Environment of RTE Meat Processing Plants: Developments, Applications and Challenges

JULIE WELLER: *Hygiena, New Castle, DE, USA*
MIKE CLARK: *Bio-Rad Laboratories, Hercules, CA, USA*
GABRIELA LOPEZ VELASCO: *3M Food Safety, St. Paul, MN, USA*

Environmental *Listeria* monitoring in the post-lethality environment is a critical process to ensure the safety of ready-to-eat (RTE) products. Typically, RTE meat processing plants swab for both food contact and non-food contact surfaces to find *Listeria species*. It generally takes up to 48 hours from the time of shipping the swabs to a third-party lab for analysis and receiving the final report. The process gets even more tedious and time-consuming when environmental swabs are positive or presumptive for *Listeria*, specifically from food contact surfaces and areas adjacent to the food contact surfaces, significantly affecting the daily operations of the RTE meat processing plants. The follow-up corrective actions including the mandatory requirements of negative findings in the three consecutive follow-up swabs are needed before the plants can go back to normal processing/production operations. The process causes significant disruptions in the operations of the plants. Therefore, rapid detection and reporting of *Listeria* within a shift would provide a major relief to the meat processors. In this symposium, some of the available rapid detection methods (within a shift) will be discussed by the manufacturers of the product. Further, the benefits and challenges of using such methods in industry applications will be explored.

S17 Global Recommendations on Food Allergens from the Joint FAO/WHO Expert Meeting

LAUREN JACKSON: *U.S. Food and Drug Administration, Summit Argo, IL, USA*
BEN REMINGTON: *Remington Consulting Group B.V., Utrecht, The Netherlands*
JOSEPH BAUMERT: *University of Nebraska-Lincoln, Lincoln, NE, USA*

For some people, certain foods may trigger an allergic reaction – a medical condition where their immune system mistakenly responds as it would to a danger. The proteins in food that trigger allergic reactions are known as food allergens. Approximately 220 million people worldwide have food allergies. A single food item may contain more than one allergen. People may have allergies to multiple foods.

Allergens in food have been considered by the Codex on a number of occasions since 1993. The list of foods and ingredients known to cause hypersensitivity was included into the General Standard for the Labelling of Packaged Foods (GSLPF) in 1999. Since then, a new paragraph relating to biotechnology (2001) and the insertion of 'Milk Protein' in the list of class names (2003) were updated within the GSLPF relating to the allergen. The Codex Committee on Food Labelling (CCFL) is reviewing provisions relevant to allergen labelling in the GSLPF as well as developing guidance on the use of precautionary allergen or advisory labelling (PAL) since 2020. The Codex Committee on Food Hygiene (CCFH) has developed a code of practice (CoP) to provide guidance to food business operators and competent authorities on managing allergens in food production in 2020. To meet the new requests of the CCFL and CCFH and support their work, FAO and WHO have decided to hold a series of expert consultations on risk assessment of food allergens.

Over the period from 2020 – 2023, the Expert Committee on this topic convened on multiple occasions and recommended the updated global priority food allergen list, threshold levels for global priority allergenic foods and also other important allergens which are not in the global priority list, precautionary allergen labelling to address unintended allergen, the exemptions of certain ingredients derived from priority allergenic foods.

This session will summarize the recommendations made from this FAO/WHO expert consultation on risk assessment of food allergens and the scientific basis for those recommendations along with comments on the timeline for Codex adoption of these recommendations and their impact on global regulatory considerations and harmonization.

S18 Understanding Consumer Reactions during Foodborne Illness Outbreaks and Food Recalls: Research from CDC, USDA, and FDA

KATHERINE MARSHALL: *Center for Disease Control and Prevention (CDC), Fort Collins, CO, USA*
AARON LAVALLEE: *U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C., USA*
FANFAN WU: *Food and Drug Administration, College Park, MD, USA*

The Centers for Disease Control and Prevention (CDC) estimates that about 1 in 6 Americans gets sick each year due to foodborne diseases. To reduce or prevent adverse health impacts associated with foodborne illness outbreaks, it is crucial to identify the source of contamination and to effectively communicate with the public. As the federal government agencies responsible for protecting the public's health, CDC, the U.S. Department of Agriculture (USDA), and the Food and Drug Administration (FDA) coordinate and share information about foodborne illness outbreaks and food recalls with the public.

Communicate effectively during such events can be challenging. Often, limited information is available at the early stage of an outbreak, with more information becoming available as the investigation progresses. Additionally, communications must be easily understandable, so that consumers can take actions to protect themselves from getting sick. To further enhance the agencies' communication with the public, CDC, USDA, and FDA conduct research to better understand consumers' awareness, knowledge, perceptions, and behaviors during foodborne illness outbreaks and food recalls.

CDC conducted a short, rapid turnaround survey in August 2023 after announcing a *Listeria* outbreak linked to ice cream. This survey assessed awareness and behavior related to the outbreak and tested two versions of the outbreak messages. USDA conducted a multi-year research project that consisted of quantitative and qualitative consumer research. Web surveys inquired about consumer awareness, perceptions, and preferences around recalls and outbreak communications. USDA also conducted focus groups to assess consumers' response to food recalls and to obtain feedback on a revised version of a USDA food recall alert. FDA recently conducted two quick turnaround consumer surveys: one during a frozen strawberries recall (fielded in March/April 2023) and one during a flour recall (fielded in May 2023). This symposium will discuss background, methods, findings, applications, and implications of the work from the perspective of each agency.

S19 Grounding the Discussion on Toxic Elements in Food: Updates from Production to Regulation

EILEEN ABT: *U.S. Food and Drug Administration - CFSAN, College Park, MD, USA*
ANGELIA SEYFFERTH: *University of Delaware, Newark, DE, USA*
EMILY MOYER: *IFPA, Washington, D.C., USA*

Toxic elements such as lead, cadmium, arsenic, and mercury are widely present in the environment from natural and anthropogenic sources. The ubiquitous presence of these elements in the environment, including in agricultural soils, results in their introduction into the food supply beginning through natural uptake of these elements into agricultural crops in the field. This symposium aims to address the critical issue of toxic element control and mitigation across the supply chain, encompassing the latest academic, industry, and regulatory perspectives. This symposium will begin with research findings on the impact of various mitigation efforts on toxic element concentrations in crops. This will be followed by a grower perspective on the opportunities and challenges of implementing control programs, and how this impacts consumer-facing risk communication. The final session will be an update from FDA CFSAN and USDA NIFA on the status and future activities of the Closer to Zero initiative including interagency collaborations and funding aimed at reducing dietary exposure to toxic elements. By connecting efforts among academia, industry, and regulatory bodies, this session endeavors to synergize collaboration and knowledge exchange, ultimately contributing to the advancement of effective and workable toxic element control measures across the supply chain.

S20 Sample Pooling: Luring or Solution?

PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*
ERIN CROWLEY: *Q Laboratories, Cincinnati, OH, USA*
ALVIN LEE: *Institute for Food Safety and Health, Illinois Institute of Technology, Bedford Park, IL, USA*

Food manufacturers are responsible for the safety and quality of their products; companies must have a food safety plan and comply with the food safety regulations. Pathogen surveillance play a critical role in this process. To ensure proper monitoring within a food production chain, sampling and testing is performed on numerous test portions and various food matrices and environmental surfaces. However, sampling is a stochastic process, and the uncertainty of results is increased by the non-uniformed distribution of microorganisms in a food batch. The current trend in the food industry is to develop an appropriate sample pooling strategy to improve the efficiency of large-scale pathogen screening campaigns by reducing the number of tests. In addition to the cost-effective approach, the goal, of course, is to better assess the risk of a foodborne hazard.

Multiple reference methods of the US administration offer large sample size testing. The International Standardization Organization (ISO) recently conducted a survey to evaluate the current pooling practices in the food industry. It appears that dry and wet pooling are more common than expected in multiple production and for different pathogens.

But how to evaluate the performance of standard or alternative methods when increasing the test proportion sizes? What are the current and globally accepted technical rules to avoid problems with inadequately validated or verified pooling methods?

Ultimately, are we confident that sample pooling is improving risk assessment given the various scenarios of the spatial distributions of batch contaminations? From usual 25 g test portion size to more than 375 g, how is that impacting uncertainty and risk assessment?

The results of the ISO survey will be presented followed by the validation of the pooling of samples by the certification bodies of rapid methods or by testing laboratories. The challenge of the final presentation will be to make pooling strategy and risk assessment easy for non-statisticians!

S21 Public Health Consequences of *Listeria monocytogenes*, and Possible Future Regulatory Approaches That Reflect a Risk-Based Approach

HILARY WHITHAM: *CDC, Atlanta, GA, USA*
DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
CRAIG HEDBERG: *UMN School of Public Health, Minneapolis, MN, USA*

Listeria monocytogenes is considered a ubiquitous and unavoidable environmental pathogen in food processing facilities. It is also a major foodborne pathogen of concern with a high mortality rate exceeding 15%, particularly among vulnerable sub-populations. Regulatory policies aimed at prevention and control of *L. monocytogenes* target ready-to-eat (RTE) foods, although the predominant global thinking is that RTE foods that support the growth of the pathogen present higher risk, and this is reflected in their zero-tolerance approach for this category. Further, these regulatory systems acknowledge all RTE foods do not present the same risk by way of a 100 CFU/g tolerance for the presence of *L. monocytogenes* in foods that do not support growth. In a departure from these global standards, U.S. *Listeria* policies apply a hazard-based compliance approach and is reflected in a zero regulatory action limit across all foods, regardless of risk. Current science and global regulations including Canada's revised 2023 *Listeria* policy reiterate the adequacy of a risk-based approach to protect public health. In recent years, academic and industry stakeholder have also called on U.S. regulators to endorse more practical and science-based policies, particularly to address *L. monocytogenes* in low-risk foods.

This symposium will bring together food safety and public health experts to lay out the public health consequences of providing a regulatory tolerance for low-risk foods in the United States.

S22 Food Packaging Should Protect, Not Hurt: Assessing and Mitigating Physical Hazards in Packaging Materials

AMANDA JONES: *Purina, Saint Louis, MO, USA*
KEITH RHOADES: *Intertek, Arlington Heights, IL, USA*
SARAH SMITH-SIMPSON: *Nestlé Nutrition, Fremont, MI, USA*

This symposium focuses upon the Physical Hazards accessible by consumers when they interact with food packaging and will shed light on how to evaluate, measure, mitigate, and ultimately communicate physical safety risks related to food packaging.

Approaches to mitigate Physical Safety often include a three staged process: first try to eliminate, then mitigate any remaining hazards, and finally communicate to consumers on potential hazards. This risk mitigation can be successfully managed through HACCP programs. The symposium will start with how to conduct a design safety analysis on food packaging. The examples and learnings presented will be on physical product brought forth to the audience in an interactive and inclusive way. Included will be identifying concerns, quantifying hazard, and then offering mitigation strategies to manage risk posed by tamper evidence, child resistance packaging, sharp edges and points, in addition to suffocation, and airway obstruction related concerns.

Further discussion will be the lack of regulations and standards to guide food packaging physical safety. Most refer to the Small Parts Legislation written for toys, yet how applicable is this for food packaging? Another presentation will discuss how sustainability efforts have impacted physical safety risks in food packaging (i.e., package size reduction, microwave safety, package material substitutions, etc.). As we strive to create recyclable packages and formats that are better for the environment, physical safety risk levels can be impacted.

Food packaging is often the first thing consumers have contact with when purchasing, transporting, preparing, and consuming food. Lastly, this speaker will discuss how consumer communication via marketing imagery, precautionary statements, and instructions on food packages can be in mitigating risk and creating liabilities. Outcomes will be shared regarding novel methods of on-pack communication that can make an impact and thus decrease the risk associated with the package.

S23 New Estimates for the Global Burden of Foodborne Disease – Where are We and Where are We Going?

YUKI MINATO: *WHO, Geneva, Switzerland, Switzerland*
MARGARET KOSEK: *University of Virginia, Charlottsville, VA, USA*
FELICIA WU: *Michigan State University, East Lansing, MI, USA*
TINE HALD: *Technical University of Denmark, Lyngby, Denmark*
BARBARA KOWALCYK: *The Ohio State University, Columbus, OH, USA*
JOHN BASSETT: *Danone, Paris, Ile de France, France, France*

The first ever estimates of the Global Burden of Foodborne Disease were published by the World Health Organization (WHO) in 2015 and have been highly influential in creating awareness of food safety as a global public health problem and shaping the food safety agenda of supranational organizations, governments, donors and industry. In 2020, the WHO was mandated to update these estimates with a target publication date of 2025. The ambition is to present annual estimates from 2010 (the reference year for the first estimates) to 2019 at country level and to expand and update these estimates on a regular basis. Data collection to inform the estimates is ongoing and first results are expected to be available in 2024. The first objective of this symposium is to inform the participants of the ongoing process to update the global estimates, how they fit in the broader WHO Food Safety Strategy, to discuss country engagement and capacity building, presented by the WHO secretariat. The second objective is to inform about interim results from systematic reviews and other data collection activities, while placing these results in the perspective of a rapidly changing global food safety landscape. Interim results supporting estimates of the global burden of diarrheal disease and liver cancer by aflatoxins will be presented including trends over time and in relation to major drivers such as climate change. As many foodborne diseases can also be transmitted by other pathways, attribution is a critical component of the global estimates and updated methodologies for source attribution will be presented. The third objective is to reflect on how these estimates have informed and will continue to inform food safety decision making by different stakeholders in the food system. This will be achieved by describing a risk ranking exercise in Ethiopia based on WHO data, and by a presentation how the WHO estimates have informed decision making on predictive microbiology in a food company.

S24 Emerging Foodborne Pathogens in Water-Associated Outbreaks: How Technology Can Assist Outbreak Investigations

CHRISTINE YU: *FDA, Laurel, MD, USA*
GERARDO LOPEZ: *University of Arizona, Tucson, AZ, USA*
VICTORIA PRUENTE: *US Food and Drug Administration, Dauphin Island, AL, USA*

Water safety and quality has a large impact on human health and consumption. Several disease outbreaks reported worldwide have been linked with contaminated water often associated with irrigation. Naturally occurring bacteria can lead to outbreaks in shellfish growing areas. The presence of emerging foodborne pathogens such as parasites, viruses, or *Vibrio* species in water-associated outbreaks warrants a better understanding of their associated public health risks. As new pathogens of concern arise, such as *V. fluvialis* and *V. alginolyticus*, it is critical that technology support outbreak investigations.

With a focus on water safety and quality, this session will delve into the advancements and challenges of the technological tools available and the generation of impactful data during these water-associated outbreaks. The speakers will begin with an overview of recent outbreak investigations, followed by an update on the recent advancements of detection and genotyping tools in viral foodborne pathogens and *Cyclospora cayetanensis* and their relationship with water outbreaks. The session will conclude with an update on the development of new real-time PCR methods to detect emerging *Vibrio* pathogens of concern.

S25 Achilles Heel in the Food Safety Programs of Food Manufacturing Plants – Evaluating Recontamination Risks

JOHN HOLAH: *Kersia Group, Bury, UK*
JOSEPH MEYER: *Kerry, Waunakee, WI, USA*
ANETT WINKLER: *Cargill, Inc., Unterschleißheim, Germany*

Recalls and outbreaks continue to occur even where a validated lethal process, industry recommended inactivation treatments, and/or regulatory guidance on adequate process interventions are being applied. Many of these pathogen contamination events, however, could be attributed to post-process recontamination. Where is recontamination introduced? Are there process steps after a post-lethal treatment, or areas interior of an equipment which can predispose a finished food product to contamination by a pathogen? Should audits of food safety programs extend beyond GMP compliance, achieving the requisite log reduction for pathogen inactivation and having an environmental program focused on non-product contact areas? What other strategies are there that could be utilized by a food manufacturer to avoid product/ingredient recontamination besides finished food testing, environmental monitoring for pathogens, increased downtime for wet and/or dry cleaning? While the establishment of an environmental monitoring program could alert a manufacturer of a potential pathogen risk in the production environment, contaminated product may already have been inadvertently released for commercial sale, if the contamination originated from niche areas interior of an equipment. By augmenting our knowledge of the process, equipment design, and operational challenges in a manufacturing facility, a proactive approach to prevention of food recontamination can be developed. This symposium will present examples and approaches how to identify and target specific risk areas based on the nature of the food product and process that it is subjected to. Incorporating these strategies in a facility's food safety initiatives can be an effective tool to enhance the safety of our food supply by effectively controlling recontamination.

S26 Food Safety within the Traditional and Modern Horticultural Sector in Africa

ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture, Abeokuta, Ogun State, Nigeria*
GLORIA LADJEH ESSILFIE: *University of Ghana, Legon, Ghana*
GENET GEBREMEDHIN: *GAIN, Addis, Ethiopia*

"Note to the Program Committee: The current proposal for a short symposium had been approved for the IAFP 2023 meeting in Toronto but none of the presenters was able to travel to Canada because our visas were not issued in time."

Managing Food Safety within the context of Food Security in Africa essentially is typically a trade-off between food safety, food security of consumers and livelihood of food producers/sellers. This trade-off exists with respect to food traded at informal/traditional markets as well as formal/modern markets. A particular area in Africa experiencing food safety challenges is the horticultural products sector, which is an important sector for food producers and consumers across the continent. Many different products and high volumes into food value chains and onto informal and formal markets day-by-day. Contamination with pathogenic and (antibiotic) resistant microorganisms can take place at different stages in food value chains, these become pertinent food safety concerns when not adequately managed. More precisely, water is used ubiquitously, for example not only as drinking water for humans and animals but also for cleaning fruits and vegetable or for irrigation. Thus, it is a key element in the food chain. Consequently, the contamination of water might affect the whole food value chain if not treated properly. Abattoir effluents of which wastewater is insufficiently managed is one reason for contamination with mixture of antibiotic resistant bacteria coming from abattoir workers and food-producing animals which is spread via the water canals. Crop irrigation, application of pesticides with contaminated water and cleaning of sale fruits and vegetables at markets with water from different sources are also primary sources for the spread of the contaminated bacteria. Some of the identified microbial and chemical hazards are *Escherichia coli*, Salmonella sp. and pesticides. Fruits and vegetables are increasingly praised as healthier foods but with little attention on the safety even though they are becoming increasingly important in the food chain and may as well have contact to potentially contaminated water from slaughterhouses. This is a challenge and requires initiatives towards understanding the situations with relevant intervention.

The aim of this session is to picture the food safety situation and challenges in horticultural production within the continent of Africa as experienced by researchers from different countries in Africa and raise a discussion on the risks and what can be done to addressed this in the local context.

S27 Complexity in Baking Process – Food Safety Challenges, Risk Management, and Validation

LAKSHMIKANTHA CHANNAIAH: *University of Missouri, Columbia, MO, USA*
REID IVY: *Ferrero North America, Chicago, IL, USA*
ANDREW ROSENTHAL: *Reading Thermal, Sinking Spring, PA, USA*

Baking is one of the oldest methods of preparing food, and the technology has been widely used to produce a variety of foods from baking bread to roasting nuts. Unlike other thermal processing methodologies, baking involves the use of specific equipment with many factors that can affect the quality of the product. In order to ensure consistency in finished baked goods, besides the recipe, one needs to understand factors such as heat source type, heat penetration, air flow, air circulation, oven damper position, etc. The current regulation requires food companies to conduct a thorough risk assessment and demonstrate that the thermal process can deliver safe foods through validation. The baking time and temperature play a crucial role in achieving end-use quality parameters and food safety. Therefore, it is critical for bakers to implement effective baking preventive

control(s) to ensure the safety of the finished bakery products and to comply with regulations. This is where validation plays a crucial role. A baking validation provides scientific proof that a baking is capable of controlling pathogenic microorganisms such as *Salmonella*, *E. coli* O121 etc. under a worst-case scenario process condition. The industry relies heavily on baking validation studies (through challenge studies and/or the use of thermal inactivation models) to achieve food safety with different recipes, optimize baking processes, and prevent foodborne illness outbreaks. The aim of this symposium is to shed light on the complexity of the baking process, how to assess and manage risks, achieve food safety, and comply with regulations. To our knowledge, the above topic has not been given a lot of attention at the IAFP annual meetings. Low-water activity PDG fully supports this symposium.

S28 From Kimchi to Kombucha: Exploring the Diversity of Fermented Foods, Understanding Preventive Control and Navigating the Regulatory Ambiguities

HAMED ZAHEDI: *Giraffe Foods (A Symrise Company), Mississauga, ON, Canada*
FRED BREIDT: *U.S. Department of Agriculture – ARS, Raleigh, NC, USA*
JULIA FUKUBA: *University of Massachusetts Amherst, Amherst, MA, USA*
JENNIFER PERRY: *University of Maine, Orono, ME, USA*
HAE WOONG PARK: *World Institute of Kimchi (Wikim), Gwangju, South Korea*

Fermentation is a traditional food preservation method that has rapidly risen in market trends due to their health benefits, novel flavors and strong consumer appeal as a "natural" food processing method. 'Properly' fermented products have a certain level of acidity to control pathogen growth which is poorly defined, and several outbreaks and recalls have revealed the microbial risks. Since the Food Safety Modernization Act (FSMA) was enacted in 2011, small and medium-sized fermented food processors are required to establish a food safety plan. Research on this field urgently needs attention as marketing potential is increasing while major concerns by consumers and regulatory agencies exist concerning safety. Validation studies are needed to provide evidence that identified process control measures can effectively control pathogen growth, however, there are limited risk assessment studies and research data that define process parameters to control the microbial hazards during the fermentation process.

This symposium addresses the current state and significance of food safety research in fermented food safety. The symposium highlights the current need to further understand the chemical and microbial changes occurring during the process that mitigates risk of foodborne illnesses. Presentations will include data supporting the identification of critical control parameters for a variety of popular fermented food products from both an extension and research perspective. Highlights will include recent data for understanding acid production rates, pathogen sensitivity to acid and the creation of predictive models for pH evolution and buffer capacity changes in popular fermented foods and beverages such as kimchi, kombucha, flavored sauerkrauts, and kefir. The data presented will help fill a major knowledge gap for producers, consumers, extension and regulatory agency personnel.

S29 Predicting the Unpredictable: How Translatable are Available Microbial Models to Risk Assessment of Plant-Based Foods?

HEIDY DEN BESTEN: *Wageningen University and Research, Wageningen, The Netherlands*
KARIN BEEKMANN: *NIZO Food Research, Ede, The Netherlands*
AIXIA XU: *ADM, Denver, CO, USA*
CHRYSANTHI CHAMPIDOU: *Nestle, Lausanne, Switzerland*

The market for plant-based foods as alternatives for dairy, fish or meat products is growing exponentially, combined with an increasing availability of novel plant-based ingredients. New plant-based product formulations and processes may introduce microbiological food quality and safety issues that were not a problem before. Prediction, assessment and mitigation of risks are complicated by a lack of data, experience and understanding of the types of microbes and their behaviour in products belonging to these relatively new food categories. Current available models on microbial growth and inactivation are based on decades of research in established food applications, such as dairy and meat. These models cannot be used directly for reliable risk assessment of plant-based alternative food products, because of different matrix compositions that influence growth or (heat) inactivation properties of microbial contaminants in these products.

In this symposium, the translatability of existing models to plant-based alternatives will be discussed, including current challenges herein. New insights on spore heat inactivation kinetics, bacterial growth, and potential safety implications resulting from microbial toxin formation in alternative foods will be presented. These data are essential to adapt and improve microbial predictive models for plant-based product applications, to overcome restrictions of currently available generic models. The industry plays an important role in ensuring the microbiological quality and safety of foods. The industrial perspective on the evaluation and use of predictive models to control the microbiological risks of plant-based foods is thus an important topic covered by this symposium.

S30 Fresh Produce Food Safety Culture Perspectives from the U.S. and Central America (The Food Industry, Government, Consumers and Schools)

SAMIR ASSAR: *FDA, College Park, MD, USA*
KEVIN ROBERTS: *Kansas State University, Manhattan, KS, USA*
ZOILA CHEVEZ: *Auburn University, Auburn, AL, USA*

This symposium will discuss the significance of human thought and behavior from three standpoints, to reduce the risk of foodborne illness associated with contaminated fresh produce. The first talk will cover Core Element 4 of the FDA's New Era of Smarter Food Safety Blueprint, *Food Safety Culture.* The speaker will address the beliefs, attitudes, and behaviors of key individuals in the fresh produce production chain, as well as the role of FDA employees and inspectors. This will include tools to assess food safety culture, education, an *FDA Food Culture Marketing Plan,* and how companies' positive food safety culture can factor into reduced inspection frequencies. This will help the industry leverage behavioral science strategies to influence executives, managers, QA personnel, and field, packing house and production employees to grow their food safety knowledge and awareness, energy, resources, and behaviors to reduce foodborne contamination risks. The second talk will cover fresh produce safety culture perspectives from Central America, addressing the speaker's home country of El Salvador in addition to neighboring countries. This lecture will address food safety issues pertaining to production, processing, import and export of fresh produce in Central America. The third talk will deal with the USDA's Food Safe Schools: Creating a Culture of Food Safety Action Guide, presented by one of its co-authors. The goal of the guide is to *Protect your students from foodborne illness by creating a food-safety culture.* Topics covered include roles that teachers, parents, school nurses and others in the school community play in food safety, a checklist and action sheets for employees to assess and address food safety goals, tips to effectively communicate food safety messages to other school community members, and how to foster a culture of food safety in schools.

S31 Climate Change: Is It Affecting the Prevalence of Foodborne Pathogens in the Environment?

MICHAEL STRICKLAND: *University of Idaho, Moscow, ID, USA*
MARTIN RICHTER: *German Federal Institute for Risk Assessment, Berlin, Germany*
YNES ORTEGA: *University of Georgia, Griffin, GA, USA*
ROBERT GILMAN: *Johns Hopkins University, Baltimore, MD, USA*

Foodborne illness are being reported annually despite the preventive and control strategies being implemented. Climate change may be affecting microbial diversity on the various food production environments resulting in changes on the persistence of current and novel food matrices. Soil health and human health are interconnected in more ways than previously recognized. Healthy soils are needed for good food production and the levels of carbon sequestration, detoxification, water, nutrient retention, and maintaining diversity will vary according to the soil type and health. Global climate changes can increase soil temperatures, affect water availability, modify soil ecology, and influence the behavior of pathogenic microorganisms, insect populations, and reservoirs. Disease causing microbes (bacteria, parasites, and viruses) in various soil types, could behave differently favoring survival, multiplication, and development of antibiotic resistance. Human behavior also influences soil health, pollution (chemical, physical, and microbial), and antimicrobial resistance among others. This symposium aims to address the environmental factors during food crop production and subsequent effects on foodborne pathogens.

S32 Modeling Everywhere: How Models Can Aid Decision Making in Food Safety and Shelf-Life Extension

SURABHI WASON: *Kerry Ingredients, Beloit, WI, USA*
NANJE GOWDA N APPANNA: *University of Arkansas, Fayetteville, AR, USA*
MATTHEW J. STASIEWICZ: *Department of Food Science & Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, USA*
MARTIN WIEDMANN: *Department of Food Science, Cornell University, Ithaca, NY, USA*

Predictive models are gaining acceptance in the food industry for risk assessment and to enhance food safety. In the literature, predictive models have been developed using data collected in both broth-based and food matrices. There are advantages and disadvantages to both approaches. Developing food specific models is challenging and impractical. Broth-based models provide worst-case scenario (over prediction – conservative estimates for food safety) and can be generalized widely instead of several food-specific models. Use of optical density (OD) measurements is rapid and can allow to collect a large amount of data with a wide range of intrinsic (pH, aw) and extrinsic (temperature, numerous selections of preservatives) factors. However, it has been difficult to translate the OD prediction to log (CFU), and for various matrices to make decisions. The symposium will be divided into two dynamic segments. The first talk will discuss an approach for data transformation, estimation of growth parameters and provide a case study that will discuss how this model can be deployed for selection of preservatives to enhance shelf life and reduce food waste. The next talk will discuss the application of predictive models to remove food safety and spoilage barriers for food recovery. Specifically showing how relatively simple predictions of virus cross-contamination or spoilage organism growth can identify ways to allow K12 schools to manage share tables in a way that would likely reduce food waste without meaningfully decreasing food safety or quality. In the final talk, we will demonstrate how predictive models can be integrated into risk assessment models, demonstrate a case study application, and explore how artificial intelligence models could be used through an application for Dairy products. Our symposium focus is to share the knowledge on practical solutions for quick development of predictive models, deployment, and their usage to reduce the risks associated with microbial contamination in foods. By showcasing the novel and best practices in food preservation, we aim to equip attendees with actionable strategies for enhancing food safety.

S33 Dry Cleaning and Sanitation in Dry, Low-Moisture Environments

LYNNE MCLANDSBOROUGH: *Department of Food Science, University of Massachusetts, Amherst, MA, USA*
ALEXIS M. HAMILTON: *Virginia Tech, Blacksburg, VA, USA*
DEBRA SMITH: *Vikan, Swindon, Wiltshire, UK*

Dry cleaning and sanitation are paramount to ensure the microbial safety of low-moisture foods (LMF). Many traditional sanitation programs utilize water and aqueous based sanitizers to control environmental pathogens. However, the introduction of water into facilities that manufacture low water activity, ready-to-eat foods, like grains, nuts and spices, could increase the potential for pathogen outgrowth and compromise the safety of the product. LMF processing relies on alternative non-aqueous based methodologies to provide effective microbial control without adding moisture back into the product. However, with current non-aqueous based sanitation protocols, there is still uncertainty regarding the effectiveness, application and occupational concerns of these alternative strategies. How can dry sanitation protocols effectively remove organic matter and microorganisms from surfaces-as effectively as water-based methods-while minimizing the risk to users?

This session aims to address these knowledge gaps related to the use of alternative non-aqueous cleaning and sanitation practices. Topics will cover dry sanitation solutions, beyond current practices, that can effectively address microbial safety in dry, low moisture environments as well as their application in industry settings.

S34 Persister: A Dormancy State of Pathogenic Bacteria in the Agro-Ecosystem and Food Supply Chain

LUXIN WANG: *University of California, Davis, Davis, CA, USA*
KEITH WARRINER: *University of Guelph, Guelph, ON, Canada*
JINSONG FENG: *Zhejiang University, Hangzhou, Zhejiang, China*

The bacterial persister state is a form of dormancy that represents a temporary resistant state to ensure a proportion of the population survives the imposition of stress. Persister cells were initially thought to result from a stochastic switch between the exponential growing cells and slow or non-growing cells. However, recent studies suggest that this epigenetic switch has a genetic basis that evolved to slow down metabolism and protect cellular structures to ensure long-term survival. The persister state has been encountered in human pathogens, such as *Listeria monocytogenes*, *Salmonella*, Shiga-toxigenic *E. coli*, and *Campylobacter* and provides not only a general survival strategy but also the potential to cause long term latent infections given that it imparts cells with temporary antibiotic resistance. There is a debate if the persister and viable but non-culturable (VBNC) states are distinct or the latter a continuum of the former. Both share many characteristics in terms of tolerance to antimicrobial agents, nutrient deprivation and thermal tolerance. A key question remaining is if dormant cells are encountered in food systems and if so, what risks they represent. This is further complicated by the lack of reliable methods to differentiate dormant cells from non-dormant cells that includes this transitioning into a non-viable state. In the following symposium a selection of leading experts in the area will provide the latest research on persister and dormant bacteria in the environment and food systems. How dormant pathogens aids survival in the food chain along with progress made in identifying agents to revive such cells will be discussed.

S35 One Health Approach to Address Zoonotic Foodborne Parasites

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DAVID CARMENA: *Spanish National Centre for Microbiology, Health Institute Carlos III, Madrid, Spain*
ROSA M. ANDRADE: *Univ of California, Irvine (UCI), School of Medicine, Irvine, CA, USA*

One Health is the cooperative effort of multiple health science disciplines to attain optimal health for humans, animals, and the environment. A One Health approach is essential to prevent the spread of foodborne and waterborne zoonotic pathogens through the food production chain. In that regard, animal health professionals play an integral role because animals both impact and are impacted by people and the environment. Parasites are frequently transmitted to humans through contaminated food and water. Zoonotic parasites (those that can be transmitted between animals and humans) such as *Cryptosporidium*, *Giardia, Ascaris, Toxocara,* or *Toxoplasma gondii* among others, have been consistently found on fresh fruits and vegetables, in water, soil, and meat and are associated with cases and outbreaks of disease worldwide. The World Health Organization estimated that the global burden of parasitic diseases attributable to foodborne illnesses (11 waterborne and foodborne parasitic diseases included) caused over 407 million illnesses resulting in an estimated of 94 K deaths and 11 million disability-adjusted life years. Due to the zoonotic nature of many of the parasites of food safety importance, a One Health approach is necessary and address the animal-human-environment interface from a food safety perspective, and to control and prevent these infections. It is critical to examine sources and routes of contamination to establish mitigating and prevention strategies. This symposium will focus on the importance of ensuring that the intrusion of parasite transmission stages into the human food chain is minimized, focusing on understanding occurrence and transmission in animals. The invited speakers will provide future research or studies required to improve control of transmission of these zoonotic water and foodborne parasites to people via food or water.

S36 Risk vs. Hazard: The Consumer Impact of Diverging Global Assessments for Safety

KRISTI MULDOON JACOBS: *U.S. Food and Drug Administration, Rockville, MD, USA*
HENRY CHIN: *Henry Chin & Associates, Moraga, CA, USA*
TIMOTHY SELLNOW: *Clemson University, Clemson, SC, USA*

In the United States (US), safety assessments for food ingredients, for example, are determined partly by the potential risk of an adverse health outcome and not solely by the hazard itself. In the European Union (EU), safety assessments focus primarily on the hazard, the actual adverse health outcome. Current consumer studies suggest that Americans' food decisions are often influenced by what's trending their ecosystem of news and information. This session will explore the impact of diverging assessments for (ingredient) safety on consumer perceptions about food safety.
For this proposed session, a case study in risk communication, will focus on current U.S. and EU safety assessments for titanium dioxide (TiO2) a color additive used in food. TiO2 is an FDA approved color additive/pigment approved for use in food, drugs, cosmetics, and sunscreen products. In 2021, the European Food Safety Authority (EFSA) determined it "no longer safe as a food additive."[i] The U.S. Food and Drug Administration (FDA) still considers it safe for use in food.[ii] What impact might these differing global assessments have on consumer perception about food and ingredient safety? Do U.S. consumers feel "cheated" or otherwise unfortunate that the U.S. employs a risk-based assessment rather than a hazard-based assessment for ingredient safety? With only 17% of American's very confident in the safety of the U.S. food supply, do we risk further erosion of consumer confidence and public trust in U.S. regulatory science and credibility of authority?
With science and risk communications as elements of consumer understanding, our challenge is to become better communicators about food, risks, and benefits. This proposed session is designed for
attendees to better understand the impact of U.S. and global safety assessments for food ingredients on consumer perceptions about safety. Speakers will discuss 1) current U.S. regulatory thinking toward risk vs. hazard assessments for ingredient safety; and 2) emerging issues impacting current thinking toward risk vs. hazard; and 3) new focus group discussions with consumers on risk vs. hazard. These discussions are captured in "Hazard vs. Risk in Perception about Food Safety: The Case of Titanium Dioxide" Sellnow et al. 2023, which will be submitted to *Food Protection Trends* for publication in 2023. Along with this session, the publication will provide additional opportunities to advance the discussion regarding risk vs. hazard as potential solutions to an emerging threat to consumer trust and confidence.

[i] https://www.efsa.europa.eu/en/news/titanium-dioxide-e171-no-longer-considered-safe-when-used-food-additive

[ii] https://www.fda.gov/industry/color-additive-inventories/summary-color-additives-use-united-states-foods-drugs-cosmetics-and-medical-devices.

S37 Novel Pathogen Detection and Enumeration Approaches for Meat and Poultry

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MARTIN DUPLESSIS: *Health Canada, Ottawa, ON, Canada, Canada*
MARCEL ZWIETERING: *Wageningen University & Research, Wageningen, The Netherlands*
KAYE BURGESS: *Teagasc Food Research Centre, Ashtown, Dublin, Ireland*
JOHN SCHMIDT: *U.S. Meat Animal Research Center, USDA ARS, Clay Center, NE, USA*
NITIN NITIN: *University of California, Davis, Davis, CA, USA*

Process control measures used for foodborne pathogen control have improved, resulting in very low numbers of pathogens detected on raw meat and poultry. For example, FSIS found that many samples contained fewer than 10 cells/g of *Salmonella* on poultry carcasses post interventions. Despite this, illnesses may occur if meat and poultry products are handled incorrectly, which can allow pathogens to grow to levels that cause foodborne illness. In addition, some pathogens cause illness even when present in very low numbers especially if consumed by vulnerable subpopulations, who are highly susceptible to infection even when very low numbers of pathogens are present. Therefore, improved methods and novel approaches are needed to detect pathogens even when they are present in very low numbers and to identify the most virulent pathogens and those that pose the highest risk to public health.

Accurate detection and enumeration methods are needed to protect public health. Improved detection, quantification and identification of the most highly pathogenic bacteria will aid in overcoming on-going issues caused by recalcitrant, highly fit, persistent pathogens. Novel approaches focused on advanced microfluidic technologies, threshold detection, and probabilistic analysis should improve the safety of meat and poultry products.

This session will provide a forum for sharing information on laboratory needs and novel approaches to detect or enumerate foodborne pathogens for meat and poultry, to improve food safety and protect public health.

S38 Global Guidance on the Use of Risk Categorization for Risk-Based Inspection Programming: Sharing FAO's Experience in Africa

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SYLVAIN QUESSY: *Université de Montréal, St-Hyacinthe, QC, Canada*
VICTORIA UCHIZI NDOLO: *University of Malawi, Zomba, Zomba, Malawi*

A national food control system (FCS) is the combination of regulatory and non-regulatory activities carried out by all responsible competent authorities, and interactions of all stakeholders to this system, to ensure that food is safe across the entire food chain. It includes preventative strategies such as risk-based inspection and verification programs and emphasizes the importance of education on food safety risks. Proper implementation of efficient risk-based inspection and oversight services ensures that inspection resources are focused on Food business operators (FBOs) representing a higher risk to public health.

Information on risk factors possibly affecting likelihood of an FBO to cause foodborne diseases (FBD) is fundamental when developing a robust risk categorization framework to support decision-making and efficient work planning by competent authorities. Data, if collected in a reliable manner and for a clearly defined purpose are a powerful tool to support decision-making. The use of digital solutions can help expand the pool of data derived from official inspection, improve data analysis and management and enhance knowledge to improve and strengthen FCS over time. Risk categorization benefits from reliable information sources as well as risk assessments and risk ranking capacities.

While risk categorization models are critical for developing countries to focus inspection activities on FBOs of higher risk, the lack of data, capacities and resources render the development and implementation of such models challenging. FAO is therefore and developing overall guidance on the use of risk categorization models to help competent authorities improve their approaches to allocate their inspection resources where the greater risks lie.

This session will present different facets of the work performed at global level, as well as showcase its application in the context of an African country, Malawi.

S39 Controlling Persistent *Listeria* in Food Retail: Honing Data Analytics for Root Cause Analysis and Intervention

MARTIN WIEDMANN: *Department of Food Science, Cornell University, Ithaca, NY, USA*
AMANI BABEKIR: *Ecolab, Greensboro, NC, USA*
JACK BURNETT: *Diversey, Inc., Cincinnati, OH, USA*

Retailers at both local and global levels are looking for new ways to integrate technology and data into their existing *Listeria* risk control programs. To do this, researchers have been working on advanced approaches to measuring and managing *Listeria* risks, and retailers want to hear about these new models and where the science can take them. This session will bring together three researchers who have been delving into improved use of data analytics to controlling *Listeria* risks. They will provide retailers with a comprehensive analytical approach for risk assessment, root cause analysis, and intervention plans to control persistent *Listeria* in a retail environment. Adapting a production facility model, relevant criteria including harborage sites, operational components, and facility features will be assessed. The analytical approach for root cause analysis and interventions will assess analytical tools including Agent-Based Model, cause-and-effect diagram, interrelationship diagram, 5-whys, machine learning, and quantitative risk assessment. During this session, the speakers will demonstrate how these analytical tools can be modified into a roadmap tailored to food retail, providing a simplified and customizable analytical approach to enhance intervention plans and prevention against persistent *Listeria* in retail.

S40 Root Cause Analysis for Non-Cultivable Foodborne Pathogens: Needs, Challenges, and Opportunities

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KALMIA KNIEL: *University of Delaware Department of Animal and Food Sciences, Newark, DE, USA*
MICHELLE DANYLUK: *University of Florida CREC, Lake Alfred, FL, USA*

In recent years, frozen berries have risen in prominence as likely foodborne vehicles of human enteric virus [hepatitis A virus and human norovirus] contamination and infection. Similarly, *Cyclospora cayetanensis*, a protozoan parasite and emerging pathogen, has been identified as responsible for foodborne disease outbreaks, particularly in fresh leafy green vegetables and herbs. While they have their differences, both enteric viruses and *C. cayetanensis* are predominantly transmitted by the fecal-oral route in association with human waste. How this occurs is varied, from poor personal hygiene practices of food handlers to sewage-contaminated waters. Unfortunately, definitive root cause analysis is rarely done for these non-cultivable pathogens, even in the case of outbreaks. This is in large part due to their generally low prevalence and heterogeneous distribution in foods, as well as the inherent challenges of testing non-cultivable organisms in food and the environment. Yet root cause analysis is critical in identification of exactly how product is becoming contaminated so as to facilitate the implementation of truly effective control measures. In short, root cause analysis is the missing link.

In this symposium, an expert speaker will provide a primer on the principles of root cause analysis in the context of non-cultivable foodborne pathogens and produce. This introduction will set the stage for the next two speakers, each of which will discuss root cause analysis strategies specifically targeting either enteric viruses or parasitic protozoa. Informed by case studies from prior outbreaks, they will cover the challenges and opportunities for performing root cause analysis for these pathogens in key at-risk produce commodities, including potential risk management approaches to strengthen produce supply chains.

S41 "Cure" What Ails You: Nitrite Alternatives in Meat Systems

KATHLEEN GLASS: *Food Research Institute, University of Wisconsin, Madison, WI, USA*
STEFAAN DESMET: *Ghent University, Ghent, Flanders, Belgium*
AARON BECZKIEWICZ: *USDA-FSIS, Washington, D.C., USA*
REBECCA FURBECK: *Kerry, Beloit, WI, USA*

The goal of this symposium is to discuss the relevance of nitrite and its alternatives as common antimicrobial agents in the meat industry. Presenters will discuss cured meat products, the use of nitrite, the history of alternative curing and nitrite alternatives, residual nitrite in meat, and factors that meat processors in various geographic areas should consider regarding replacing nitrite in their formulations. A detailed overview of consumer preferences in other regions as well as regulatory hurdles will be discussed to provide a comprehensive overview of the status of nitrite alternatives to maintain food safety.

Symposium attendees will learn:

1) Efficacy of nitrite and non-nitrite alternatives to inhibit the growth of bacteria; including appropriate application levels for specific processes, as well as targeted microorganisms based on nitrite or nitrite-alternatives.

2) Regulations and use of nitrite and nitrite alternatives in the US/EU: An overview of the status of regulations and applications for nitrite and nitrite alternatives in the US and EU. Countries in the EU are implementing policy changes while others surmount it as part of their culinary tradition.

3) Dynamics of alternatives to purified nitrite: Considerations regarding replacing nitrite in the meat industry, the effects of nitrite alternatives on various meat products, regional policies related to nitrite alternatives, as well as challenges and recommendations for sourcing nitrite alternatives.

S42 Under the Influence: Impact of Plant Metabolites on Survival and Persistence of Foodborne Pathogens

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SHIRLEY MICALLEF: *University of Maryland, College Park, MD, USA*
JERI BARAK: *University of Wisconsin-Madison Food Research Institute, Madison, WI, USA*

Foodborne pathogens such as *Salmonella enterica*, Shiga-toxin producing *Escherichia coli* and *Listeria monocytogenes* can persist in agricultural matrices. Plant surfaces that come in contact with contaminated soil or water can harbor these microorganisms. Foodborne pathogens have specific virulence factors to evade host immune responses, survive and proliferate in human or animal hosts. Pathogens sense and adapt to the prevailing conditions by modulating their gene expression on a global scale. It is well established that factors such as temperature, iron, metal ions and pH in animal hosts can trigger the transcriptional activity of virulence genes or operons in foodborne pathogens. Similarly, the expression of genes for virulence, persistence, stress adaptation and many other vital processes can also be regulated by molecular signals and metabolites found in plants. While some secondary plant metabolites have antagonistic activity against foodborne pathogens others might promote growth, biofilm formation and resuscitation from stress. Plants have evolved systems analogous to animal innate immunity that recognise pathogen-associated molecular patterns (PAMPs). Recent studies have indicated that presence of foodborne pathogens on plant surface can induce plant immune responses and defense mechanisms such as the production of Reactive Oxygen Species (ROS). Further, secondary metabolites from plants can induce biofilm formation among foodborne pathogens and result in higher recalcitrance to interventions such as washing and sanitation. The objective of the proposed symposium is to present research findings on the effects of plant metabolites on the persistent survival of foodborne pathogens on leafy greens, fruits and vegetables. Understanding the influence of plant metabolites on foodborne pathogen survival and persistence can help in improved risk assessment and help in the development of innovative approaches to curb produce contamination.

S43 Integrated Modeling Approaches to Support Firm-Level Decision Making in Produce Safety

KALINDHI LARIOS: *University of Florida, Gainesville, FL, USA*
CLAUDIA GANSER: *University of Florida, Gainesville, FL, USA*
MATTHEW J. STASIEWICZ: *Department of Food Science & Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, USA*

Foodborne disease outbreaks involving leafy greens continue to occur despite increased industry efforts to understand and manage key factors that affect the microbial safety of these products and increased regulatory efforts to provide a flexible but effective legal framework for produce safety. The dynamics of pathogens in leafy green production are highly complex involving nonlinear processes with many interacting factors. The impact of these factors varies between production locations, depending on climate, soil composition, field management, surrounding land use, wildlife intrusion, food processing parameters etc. A critical factor for one site may not be relevant for another site. Moreover, produce contamination events are rare but may have major impacts. We believe that to fully understand the complex dynamics and interacting factors of pathogens in produce production, a suite of dynamic and stochastic farm to fork models needs to be developed, allowing site-specific predictions of risk factors and impact of management options on consumer risk. The NIFA-funded CONTACT project involves a consortium of researchers and extension specialists from 9 land grant universities and one federal agency, aiming to form a systems approach applicable to different food-pathogen combinations to support produce safety decisions. This symposium will highlight efforts to develop integrated simulation models to support this aim, focusing on leafy green production, processing, distribution, and consumption, and will discuss when such models can be useful for supporting decisions by produce growers and processors.

This symposium is complementary to the proposal "The Required Evolution of Best Practices Based on Science for Fresh Cut Produce" (Submission # 9467) and both sessions would benefit from being planned back to back.

S44 Food Safety Risk Assessment in Latin America: Successful Stories from Countries Transforming Industry Standards and Food Safety Policy

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ANGÉLICA GODÍNEZ-OVIEDO: *Universidad Autónoma de Querétaro, Querétaro, QA, Mexico*
INES MARTINEZ: *Technological Laboratory of Uruguay (LATU), Montevideo, Uruguay*

Latin America is a net food exporter. From agricultural commodities to finished products ranging from coffee to meat products, countries in Latin America have been using risk assessment to address domestic and international trade food safety issues. This session will include speakers from government agencies and academia in Latin America presenting successful examples of using risk assessment to inform regulations and ultimately risk management decisions. Case-studies will include environmental chemical contaminants in coffee in Costa Rica and rice in Uruguay, sweeteners in Chile, *Salmonella* in poultry in Mexico and the lessons learned by Colombia as the first country in Latin America that created a risk assessment agency. At the end of the symposium, a round table will be held among the speakers to share their challenges and opportunities of using risk assessment for national food safety decision making.

S45 Impact of Effective and Timely Communication of Relevant and Complex Scientific Data to Influence Human Behavior

JAMES DOYLE: *Creme Global, Dublin, Dublin, Ireland, Ireland*
FRANK YIANNAS: *Smarter FY Solutions, Bentonville, AR, USA*
CATHERINE DAVIDSON: *Sabra, Richmond, VA, USA*
CHERYL BURN: *Kerry Group, Beloit, WI, USA*

Proven scientific data analysis, data integrity & data accessibility along with effective and timely communication to make sound food safety decisions is a key success criterion for food safety professionals in academia and industry. Food Safety Professionals heavily rely on effective and timely food safety data communication to make short and long-term decisions that can potentially be a matter of life and death. The collection of raw data and its accurate and timely analysis and communication are the ultimate life cycle in understanding and behavior for all stakeholders namely consumer, customer, industry professionals, and the broader scientific community.

Having said this, the effective and timely communication of proven scientific and food safety data is a much bigger challenge today than ever before. Today, food industry employees, consumers, customers, and scientists have access to more information from more sources than ever before. Attempting to manually extract insights from this raw data potentially brings more confusion and misunderstanding and/or misinterpretation of proven scientific data. This leads to lack of consensus with regards to source and/or interpretation of scientific data, ineffective and untimely communication of relevant information and, eventually no positive behavioral change.

This symposium is intended to discuss and review examples from Industry, Regulatory and Consulting point of view on how to best communicate proven scientific food safety data effectively and in a timely manner to impact behavior as intended. There are several ways accomplish this task, however the goal is to present the audience with examples of best practices for effective and timely food safety data communication.

S46 Bringing the Environment into the Lab: Preventing the Next Outbreak by Using Controlled Environments to Understand What Caused the Last One!

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KALMIA KNIEL: *University of Delaware Department of Animal and Food Sciences, Newark, DE, USA*
SANJA ILIC: *The Ohio State University, Columbus, OH, USA*

Many experimental studies conducted in a laboratory setting have been successfully implemented in the field, from antimicrobial and pesticide treatment to genetic manipulations of plants for various beneficial reasons, such as resistance to plant pathogens and insects, enhancement of fruit flavor, nutrition content, etc. There is a rich, lengthy, and geographically-diverse history of using controlled environments for growing plants and produce, with records of this practice dating back to the Roman Empire. Food safety researchers are now using controlled environments, such as growth chambers, to provide an understanding of factors such as environmental temperature, humidity, soil type, soil microbiome, etc., that may Influence microbial survival, uptake by the plant, proliferation, as well as control of microbial pathogens in water, soil and plants, in the growing fields. It is almost impossible for many of these studies, particularly, the ones involving pathogens, to be conducted in the open fields; controlled environments, such as growth chambers, allow us to follow the contamination of plant from seed to harvest during the growing period. In this symposium different studies conducted in "the lab" using environmental information from the growing field environment will be described.

S47 Low Calorie Sweeteners: An Update on the State of the Science

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MAIA JACK: *American Beverage Association, Washington, D.C., USA*
KRISTI MULDOON JACOBS: *U.S. Food and Drug Administration, Rockville, MD, USA*

Regulatory agencies, including the U.S. FDA, have continued to determine low-calorie sweeteners to be safe, building on an extensive dataset that demonstrates their safety. Despite that, consumers continue to have questions about the role of these substances in a wide range of products. Recent controversy about low-calorie sweeteners presents an excellent opportunity for food toxicologists to educate the public about the extensive processes in place to evaluate the safety of food ingredients, as well as highlight important concepts that are key to food chemical risk assessment. This session will use low-calorie sweeteners to examine the processes that industry and government undergo to establish food ingredient safety. The speakers will also describe the fundamental concepts of hazard and risk in the context of food safety assessment. These fundamental concepts will also be explored through an exploration of the evaluations of one low-calorie sweetener (aspartame) conducted by two prominent organizations, the Joint Expert Committee on Food Additives (JECFA) and the International Agency for Research on Cancer (IARC), as well as how the decisions by these agencies impact regulatory decision-making.

S48 From Pathogen Transcriptomics to Prevention Strategies

XINYI ZHOU: *Illinois Institute of Technology, Bedford Park, IL, USA*
SULTANA SOLAIMAN: *University of Maryland, College Park, MD, USA*
LUXIN WANG: *University of California, Davis, Davis, CA, USA*

Next-generation sequencing (NGS) is a powerful tool that can be applied to food safety research in myriad ways. Advances in NGS and bioinformatic methodologies are paving the way for more robust research to elucidate the mechanisms used by bacterial foodborne pathogens to survive in foods, during food processing, and in the food processing environment. Transcriptomics, specifically RNA-sequencing, is a commonly used tool to uncover the differential gene expression and gene regulation of pathogens in response to stress. Enrichment of cellular, metabolic, and biological processes can subsequently be inferred from these results. Often, research presented in the published literature ends with a description of these enriched pathways; no application of the results to relevant food safety issues is offered. The goal of this symposium is to close the loop between transcriptomics research and the application of the findings to real-world scenarios. Speakers from both academia and government will present their transcriptomic research and give examples of the applications of their research, ultimately leading to prevention strategies.

S49 Foodborne Pathogen Biofilms, Environmental Microbial Community, and Food Safety

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XIANGWU NOU: *U.S. Department of Agriculture–ARS–BARC, Beltsville, MD, USA*
SAPNA DASS: *Texas A&M University, College Station, TX, USA*
XIANQIN YANG: *Agriculture and Agri-Food Canada, Lacombe, AB, Canada, Canada*

Biofilms are surface-attached microbial communities embedded in extracellular substances that protect the bacteria from physical or chemical stress. Many foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella enterica,* and *Listeria monocytogenes* can persist in the environment and survive for prolonged periods of time via biofilm formation. Therefore, biofilms pose a serious food safety risk by harboring pathogens and causing food contamination, a reduction in product quality/shelf life, and potential disease transmission. Furthermore, available studies have demonstrated the critical impact of environmental microorganisms on pathogen survival and prevalence via mixed-species biofilm formation. The synergistic or antagonistic interactions among the resident microflora can either promote or inhibit the colonization and survival of specific pathogens in the mixed biofilms. The various pathogens may also apply multiple strategies, either as individual strains with unique intrinsic properties or interacting with environmental microorganisms, to tolerate stress, outcompete, and/or coexist with environmental companion bacteria for extra protection, leading to higher survival capability. Meanwhile, the persistence of non-pathogenic spoilage communities in food facilities, often as biofilms, may result in microbial spoilage which is the major cause of food waste. Thus, the importance of biofilms in food safety has attracted significant attention from academic, regulatory, and industrial fields in an attempt to understand the real impact and functional mechanisms of bacterial biofilms. To that end, novel experimental methods and research tools have been developed. This symposium focuses on recent studies regarding the impact of the different foodborne pathogen biofilms on various types of food products including meat, fresh produce, and dairy products. Research on the core surface-associated microbiome and the microbial ecology of food processing environments will be presented. We will also discuss the complex pathogen-environmental biofilm interactions that may correlate to potential environmental keystone species that are critical for pathogen prevalence and survival. The newly developed biofilm research method for direct visualization and investigation of pathogen spatial distribution within multispecies biofilms will be introduced.

S50 Enhancing Consumer Protection: Proactive *Salmonella* Serotyping with Data-Enriched Insights and Unified Efforts in Policy, Industry, and Biotech

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SARAH SORSCHER: *Center for Science in the Public Interest, Washington, D.C., USA*
LOLA CRESPO: *Aviagen Inc., Huntsville, AL, USA*
RAMIN KHAKSAR: *Clear Labs, San Carlos, CA, USA*

Salmonella infections constitute a significant global health challenge, imposing considerable burdens in terms of illness, death, and economic impact. In the ongoing efforts to mitigate the prevalence of *Salmonella*-related illnesses and bolster food safety, *Salmonella* serotyping has emerged as a crucial tool. Risk analysis studies have identified *Salmonella* serotype as a crucial factor that can help differentiate strains with a higher propensity to infect humans, greater virulence, or heightened antimicrobial resistance. By honing in on strains most likely to cause illness, serotyping holds the potential to substantially reduce the incidence of *Salmonella*-related illnesses and improve the safety of the entire food supply chain.

Recent developments in molecular technologies have catalyzed a pivotal shift in our capacity to easily provide a deeper characterization of *Salmonella*, including serotyping information. However, bridging the gap between laboratory innovation and real-world implementation in the food industry poses challenges. Despite the allure of risk-based strategies and advanced detection technologies, obstacles like regulations, resource limitations, resistance to change and validation hurdles hinder their integration. A targeted amplicon-based next generation sequencing (tNGS) approach may help further reduce the barrier for entry as compared to other methods. Amplifying target genes of interest using tNGS will significantly shorten turnaround time due to the ability to use enrichment cultures as starting material instead of the need for culturing of bacterial isolates. With faster turnaround time, we believe the adoption rate for *Salmonella* serotyping will improve overtime and provide actionable intelligence for the food industry.

This symposium, therefore, emerges as a platform to catalyze collaboration by convening policy makers, industry stakeholders, and method developers to cultivate an open dialogue, share experiences and enhance partnerships in order to translate these shared insights into actionable practices for *Salmonella* serotyping and increased consumer protections. It is only through this collective effort that we can mitigate the risk of *Salmonella* throughout the supply chain and reshape the narrative of food safety.

S51 Analytical Challenges in Developing Successful Risk Management and Control Monitoring Strategies

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JANELL KAUSE: *USDA/FSIS, Manassas, VA, USA*
CATHARINE CARLIN: *Mérieux NutriSciences, Chicago, IL, USA*
RACHEL BINET: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, Office of Regulatory Science, Division of Microbiology, College Park, MD, USA*

When it comes to food safety, there is no such thing as zero risk.

Microbial Risk Assessment evaluates the effectiveness of control strategies to reduce the risk of foodborne illness from pathogens in food to an acceptable level, while zero-tolerance approaches set the lowest limit for specific pathogens. Regulators usually balance between the two concepts to set-up regulation requirements.

On the other side, food business operators are responsible of developing appropriate control monitoring program to comply with zero tolerance or quantitative limits legislation. These programs combine sampling and testing process to assess the effectiveness of the contamination control measures in production plants.

Producing trusted data is one of the bottlenecks to developing both efficient risk management strategy and control monitoring of production chains. The rise of molecular techniques offers alternatives to conventional methods that have limitations for accurately enumerating low contamination levels, for rapid serotyping or for screening virulent strains. Beside the multiple features of these new techniques, their performance characteristics should be properly assessed for proper use by risk assessors and end-user laboratories.

The first talk will address the challenges of analytical uncertainties and how they balance public health priorities with reasonable business impacts. There are pros and cons for molecular analytical confirmation and typing from enrichment broths: let's get a 360° overview to support decision-making in control programs. Finally, the last talk will focus on targeting relevant virulence markers to identify, prior to isolation, STEC strains with severe health risks.

S52 *Listeria monocytogenes* in Ice Cream Products – Review of Outbreaks and Prevention Activities

AMANDA CONRAD: *Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*
MATTHEW DOYLE: *US Food and Drug Administration, College Park, MD, USA*
TIMOTHY STUBBS: *Innovation Center for U.S. Dairy, Rosemont, IL, USA*

This session will have three parts. CDC will provide a review of the epidemiology and notable attributes of listeriosis outbreaks linked to ice cream to date. FDA presentation will describe prevention activities focused on resource development and communication to ice cream producers with a particular focus on small and artisanal producers. Lastly, the Innovation Center for U.S. Dairy will discuss resources, trainings, and tools developed to assist ice cream manufacturers in making a safe product.

Since 2014, seven listeriosis outbreaks have been linked to ice cream products and milkshakes. These outbreaks have resulted in 54 illnesses, 53 hospitalizations, and 10 deaths. Prior to 2021, illnesses associated with ice cream were among persons aged 65 and older and those with immunocompromising conditions. In recent outbreaks there have been 8 illnesses among pregnant people or newborns, and one illness in a pregnant mother resulted in fetal loss. Some of these outbreaks have been limited to a short period with just two illnesses, and others have spanned over five years. Taking what is learned from the outbreaks, FDA is working on the development and implementation of a prevention strategy to enhance ice cream safety. As part of this strategy, FDA is working with industry and partners to develop and implement deliberate approaches to help promote food safety culture and limit or prevent future outbreaks linked to ice cream. The Innovation Center for U.S. Dairy will discuss the various tools they have developed and how they have leveraged their resources within the ice cream industry to change. This session will provide the status of efforts, welcome additional input, and encourage engagement.

S53 The Past, Present, and Future of Surrogates for Validating Food Safety Controls

TIM BIRMINGHAM: *Almond Board of California, Modesto, CA, USA*
IAN HILDEBRANDT: *Michigan State University, East Lansing, MI, USA*
ABIGAIL B. SNYDER: *Cornell University, Ithaca, NY, USA*

Surrogates have become an essential tool for validating preventive controls for the risk reduction of the surrogate's counterpart, the pathogen of concern. Because of the application flexibility of surrogates, they can be used to validate processes that are considered too dynamic or complex for alternative validation methodologies, such as predictive models or safe harbors. Processes treating low-moisture foods benefit greatly from surrogates, as they often involve dynamic temperature, moisture, and form. Methodologies for utilizing pathogen surrogates for process validation are not yet optimized or broadly standardized, with most of what we know of pathogen surrogates having been developed since the advent of FSMA and the Preventive Control rules. Surrogates have defined how some industries, such as the U.S. almond industry, approach validating preventive controls. In response to the ongoing demand for expanding the applicability of surrogates, research studying surrogates (most notably *Enterococcus faecium*) have rapidly expanded – and not just for validating "kill-steps." Therefore, this symposium will present the pathways that have led to the current state-of-practice for utilizing surrogates for process validations and describe a preferred future for optimal utilization of these essential tools. The speakers will address 1) how *Enterococcus faecium* has become the surrogate of choice for low-moisture foods (and more), 2) how the current state of surrogate utilization can be improved for researchers and processors, and 3) the untapped potential of surrogates for validating other processes of concern within the food industry.

S54 Can a One Health Approach be a Roadmap to Reduce Salmonellosis?

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One Health is a concept that embraces the interconnectedness of the health of humans, animals, and the environment. Given the food safety and security challenges across production systems, One Health is an integrated approach to identifying solutions and responses to persistent food safety challenges. According to USDA-FSIS, while the prevalence of *Salmonella* contamination in regulated poultry products decreased by more than 50 percent in recent years, the number of outbreaks related to poultry increased, and there has been no reduction in human illnesses attributable to poultry. A U.S. interagency panel attributed almost 78% of salmonellosis cases to nine food commodities, leading to more than one million cases in the U.S. alone.

Controlling *Salmonella* across food systems is difficult. *Salmonella* can colonize the chicken gastrointestinal tract and generate a tolerogenic response by the immune system. This suppresses the immune response in chickens and allows *Salmonella* to persist as part of the chicken microbiome.

Salmonella is unique compared to other zoonotic bacteria as it cycles through host and non-host environments. *Salmonella* can be considered the epitome of a One Health challenge and is also considered a sentinel microorganism for antimicrobial resistance because of its presence in various settings. As more public health programs incorporate a One Health approach to monitoring and control, *Salmonella* prevalence in water and non-host environments can provide critical data to improve food safety outcomes.

One Health Programs have been implemented internationally on different continents. Challenges remain in establishing monitoring programs, but collected data are critical to populating databases that can provide international resources and tools to control *Salmonella*.

This symposium will provide the following to the audience: biological reasons why *Salmonella* is difficult to control in hosts; how environmental surveillance programs aid in monitoring and providing control strategies for *Salmonella*; efforts and One Health approaches across the globe that can be implemented in settings that transcend international borders.

S55 Improving Food Safety in Traditional Food Markets: The EatSafe Approach

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Food safety is a newly recognized tool for improving nutritional outcomes for development projects. Traditional food markets, a crucial source for fresh and prepared foods for the majority of consumers in Africa, often sell a wide variety of fresh foods, such as fruits, vegetables, meat, fish, cereals, and legumes. They present unique food safety challenges as they lack WASH infrastructure, resources and government support. EatSafe, Evidence and Action Toward Safe, Nutritious Foods, a Feed the Future Initiative, will report on its testing of seven food safety interventions in Nigeria and Ethiopia to stimulate consumer demand and behavior changes to improve food safety in those local markets.

The EatSafe project has demonstrated high interest among market actors in participating in activities to improve food safety, including communications initiatives; in-market activities (training, branding); and stakeholder engagement and advocacy. A market assessment informed the choice of interventions tested in each market. The impact of the interventions was derived from qualitative and quantitative assessments.

Speakers will report on the outcomes from this 5-year USAID funded intiative, and discuss the prospects for sustainable solutions to improve food safety in traditional food markets. Note to reviewers: We may replace a GAIN speaker with a speaker from Busara Center for Behavioral Economics, depending on travel funding

S56 A Summary of Recent Consumer Food Safety Behavior Research: Takeaways, Challenges, and Next Steps

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IAN YOUNG: *Toronto Metropolitan University, Toronto, ON, Canada, Canada*

Numerous research studies have been conducted by academia, industry, and nonprofit organizations and through government-sponsored studies over the past 5 years that have explored consumer knowledge, attitudes, and behaviors regarding food safety. This research includes nationally representative surveys of consumers; observation studies in test kitchens to observe participants' actual vs. reported behaviors; eye tracking studies to understand consumers' use of food safety information on food labels; and formative research such as focus groups and in-depth interviews. Through this research we have learned about consumers' adherence to recommended safe handling practices when preparing meals at home regarding the four basic practices of clean, separate, cook, and chill. Formative research has enriched our understanding of the barriers and facilitators to following these recommended practices and potential drivers to motivate behavior change.

Findings from this research suggests that there have been improvements in consumer behaviors over the past 5 years, for example, food thermometer ownership and use has increased. Despite these improvements, more work remains to be done to ensure that consumers are aware of the recommended practices and that effective messaging and dissemination methods are used to motivate consumers to change their behavior. The purpose of this symposium is to present findings from recent consumer research and to engage the speakers in a discussion about how the research findings can help inform food safety educators' efforts going forward to help reduce foodborne illness from food prepared at home.

S57 Focusing on Foodborne Illness: The Science Supporting U.S. Department of Agriculture's Proposed *Salmonella* Framework

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JOANNA ZABLOTSKY-KUFEL: *USDA Food Safety and Inspection Service, Washington, D.C., USA*
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The U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) is considering a new strategy to control *Salmonella* in poultry products and more effectively reduce foodborne *Salmonella* infections linked to these products. The Framework under consideration is science based and data driven, shaped by extensive information-gathering and discussions with a wide range of stakeholders, researchers, and scientists.

To support its Framework, FSIS sought advice from the National Advisory Committee on Microbiological Criteria for Foods (NACMCF). The Committee included its advice in a report on enhancing *Salmonella* control in poultry products (*Journal of Food Protection*, 84 (February 2024), 100168). FSIS reviewed and summarized the current science and prepared an independently peer reviewed report, "Risk profile for Pathogenic *Salmonella* Subtypes in Poultry," to inform FSIS on whether certain subtypes of *Salmonella* can be considered as adulterants in specific poultry products. The Agency also prepared two independently peer reviewed quantitative microbiological risk assessments – one on Chicken, one on turkey – that incorporate advanced analytics and genomic data. FSIS also evaluated expanded testing of poultry products and explored advanced laboratory testing and methods.

At this symposium FSIS will share information on the scientific support for the agency's *Salmonella* Framework for poultry with food safety professionals representing industry, academia, consumer organizations, the general public, and international community.

S58 From Label to Table: Understanding the USDA's Bioengineering Labeling Rule

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EVA HURT: *The Coca-Cola Company, Atlanta, GA, USA*
TAMEKA CARR: *Kroger, Atlanta, GA, USA*

What's the difference between organic apples and BE apples?....It's rDNA is CRISPR! Most people wouldn't understand the hilarity of that joke, because there's still so much uncertainty around BE and the USDA's BE Labeling Rule, but this session aims to clarify some of the confusion.

Bioengineered (BE) foods, previously referred to as genetically modified (GM) foods, have been around for centuries and though many institutions (American Medical Association, the National Academy of Sciences, WHO) have all endorsed the concept that BE foods are "safe" for consumption, a large portion of consumers remain skeptical.

Due to public outcry, states like Vermont started taking things into their own hands and creating mandatory GMO labeling laws. In order to prevent 50 different labeling laws, the Agricultural Marketing Act of 1946 was amended on July 29, 2016, with Public Law 114-216, to require USDA to establish a national, mandatory standard for disclosing any food that is or may be bioengineered. The Standard requires food manufacturers, importers, and certain retailers to ensure bioengineered foods are appropriately disclosed. This symposium is a must for anyone wanting to learn more about the updates to the BE Standard and how the Standard has effected industry and consumers.

In order to gain a better understanding about the rule and how companies are complying, we'll hear from three different perspectives:

- The USDA will give us an overview of the Standard, including the definition of a bioengineered food, updates to the BE Standard, three ways that regulated entities can demonstrate that their food product does not contain detectable modified genetic material, disclosure options, and recordkeeping requirements. Lastly, discuss the quantity and nature of BE public complaints.
- The Coca-Cola Company will detail how they use the "Digital/electronic disclosure" with QR codes and tell us the challenges associated with implementing this change.
- The Kroger Company will disucss how they've used an "On Packaged Statement" and explore their journey to compliance.

S59 From Cart to Kitchen: Data-Driven Insights on E-Commerce Food Safety for Delivery

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DONALD W. SCHAFFNER: *Rutgers University, New Brunswick, NJ, USA*
STAN OSUAGWU: *Home Chef, Chicago, IL, USA*
ANGELA SANCHEZ: *Industry, Nashville, TN, USA*
AKHILA VASAN: *Uber, Chicago, IL, USA*
SARA STARCK: *Industry, Arlington, VA, USA*

E-commerce has revolutionized how consumers shop for food, offering convenience and accessibility like never before. In this symposium, we will delve into the critical aspect of food safety within the e-commerce landscape, focusing on data-driven insights and strategies to ensure a safe and secure delivery process from the cart to the kitchen.

As responsible players in the e-commerce industry, we strive to do the right thing by prioritizing food safety across the entire supply chain. By embracing robust controls, chill chain policies, and other analytical insights, we can effectively reduce risks and uphold our commitment to delivering safe food to consumers. We will cover how we implemented the Conference for Food Protection (CFP) Direct To Consumer and Third Party Delivery guidelines.

Furthermore, we recognize the importance of last-mile controls, particularly in the context of delivery drivers. Rigorous vetting and training programs are pivotal in ensuring that our delivery personnel adhere to strict food safety protocols, guaranteeing the integrity and safety of the products throughout the delivery process. To continuously enhance our food safety efforts, we emphasize the significance of customer feedback monitoring. By actively collecting and analyzing consumer feedback, we can identify potential areas for improvement and promptly address any food safety concerns raised, enhancing trust and transparency in our operations.

Finally, we will explore the power of data mapping in our pursuit of e-commerce food safety excellence. By leveraging data analytics and mapping tools, we can gain invaluable insights into potential risk areas, enabling us to implement targeted preventive measures and maintain a high standard of food safety throughout the delivery journey.

Join us in this engaging symposium, where we will leverage data-driven insights and explore innovative strategies to ensure the highest levels of food safety in e-commerce.

S60 Rapid Microbiological Test Methods – Are They Still an Important Part of a Food Processor's Food Safety Program?

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PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
ROBERT DONOFRIO: *Neogen Corporation, Lansing, MI, USA*

There has been debate in recent years about the growth of Rapid Microbiological Methods (RMM) in food safety and, most recently, debates about whether they are still needed. With laboratory outsourcing at an historic high, and better shipping methods getting samples to labs ever faster for rapid turnaround times, and a high percentage of those samples being analyzed using definitive and reference methods, are RMM – especially those employed in-plant – still a necessary technology? Or is there still a case for the ease of use and faster time to results provided by RMM that makes for better in-plant food safety decisions?

We surveyed >250 food processors from 55 countries to find out more about RMM use in their food-safety program. When RMM were first adopted, tests were used in in-plant labs. But pathogen outsourcing has caused fewer companies to have a lab. Our survey showed that 78% (U.S.) and 54% (international) processors no longer have an in-plant lab. RMM remain popular with those processors who still have an in-plant lab, with a PCR (~55%) and instrument-based ELISA (40%) as the most used formats. Companies select RMM for faster results, with most companies now using RMM primarily to guide their environmental monitoring (EM) and hygiene verification programs. With production (not lab) employees using the tests, 75% (U.S.) and 60% (international) processors indicated "easy-to-use" as the 2nd most important attribute of RMM. Companies reported using many easy-to-use rapid indicator swabs and rapid ATP swabs, with the latter used by 76% U.S. and 59% international companies, respectively. But is faster better? Most companies indicated that < 24 h. TAT "useful," with 8h. "Nice to have" but few indicated < 8h was needed.

The symposium will discuss results of this survey, review best practices, and explore the use of RMM in context of current and future food industry testing needs.

S61 Wax On Wax Off: Foodborne Pathogen Contamination from Wax Application and Wax Applicators

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FAITH CRITZER: *University of Georgia, Athens, GA, USA*
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Fruits and vegetables are protected by a lipid envelope called the cuticle. The cuticle consists of cutin and waxes. The waxy cuticles of fruits and vegetables are hydrophobic in nature and play an essential role in preserving moisture. Moisture loss in fruits and vegetables can affect the quality of the product. Post-harvest activities such as washing, use of surfactants and brushes to clean the surfaces of fruits results in the loss of fatty acids and waxes from the cuticle. As a substitute, natural or petroleum derived waxes are applied to the surface of fruits and vegetables to reduce water loss due to transpiration and to improve the appearance of the produce commodity due to higher gloss. The impact of waxing on the microbiological safety of fruits and vegetables requires better understanding as many produce commodities such as tomatoes, peppers, and cucumbers are coated with wax post-harvest after washing. The commodities have also been associated with *Salmonella* contamination resulting in outbreaks and recalls. Recent research has indicated that *Listeria monocytogenes* could not only survive on the surfaces of waxed apples but also increase in number. Foodborne pathogens can survive on waxed produce surfaces for extended durations. Several factors such as the type of produce, brush material, wax composition and brush sanitation could be instrumental in the contamination of waxed fruit. The speakers of the symposium will present research highlighting risk factors and mitigation strategies to improve our understanding of pathogen cross transfer and survival on waxed fruit and vegetables.

S62 Food Safety and Regulatory Considerations for Raw Pet Foods: Challenges and Opportunities

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JASMINE KATARIA: *Kerry, Beloit, WI, USA*
JANAK DHAKAL: *University of Maryland Eastern Shore, Princess Anne, MD, USA*

With the increasing trend of humanization of pet food, the pet food industry has witnessed a remarkable surge in the popularity of alternative formats such as raw, freeze-dried, air-dried, gently cooked, and fresh pet foods. These minimally processed options offer a more natural approach to pet nutrition, aligning with the growing trend of humanization in pet care. Raw pet food, lacking an established kill step, brings greater food safety challenges due to the risk of growth and survival of pathogenic and spoilage microorganisms, posing potential risks to both humans and pets alike. This symposium highlights the necessity of enhancing consumer safety and shelf life of raw pet food using different decontamination technologies or through the potential incorporation of antimicrobials.

Symposium attendees will learn about: a) distinctive characteristics of these new pet food formats, exploring their ingredients and processing steps, potential pathogen mitigation strategies (including HPP, freeze-drying) as well as the critical roles and responsibilities of all stakeholders along the supply chain, emphasizing their collective efforts to enhance the safety of these products; b) food safety and spoilage challenges associated with these demands while offering insights into potential solutions and sustainable approaches for preservation of raw pet food; c) lastly, the complex landscape of pet food safety regulation, highlighting the contrasting roles of the US FDA and USDA in the U.S., while examining U.S. jurisdictions and responsibilities in ensuring the safety of pet food, providing clarity to industry professionals and pet owners alike.

This session will empower stakeholders in the pet food industry and pet parents with the knowledge and strategies needed to navigate the evolving landscape of alternative pet food formats and the importance of regulatory governance to foster safer, healthier choices for pets and their human companions.

S63 Flour: Fostering Food Safety – Industry and Regulatory Collaboration to Minimize Health Risks in Raw Flour Products

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JULIANY RIVERA CALO: *Ardent Mills, Denver, CO, USA*
ALEXANDRE PANCHAUD: *Nestlé USA, Solon, OH, USA*

Despite efforts to educate consumers on the risks, raw wheat flour and products continue to be an important vehicle for foodborne illness. Recent outbreaks and recalls linked to shigatoxin-producing *E. coli* (STEC) and *Salmonella* are indicators that more must be done by all stakeholders throughout the supply chain to improve the microbiological quality and safety of flour and flour-related products. The majority of U.S. domestic flour produced is used in commercial operations to produce flour-containing products, where expectations exist for preventive controls designed to reduce microbiological risk to consumers while at the same time matching consumer's taste expectations. It is here where the greatest overall impact on risk exposure to consumers by raw flour can be made. The exploration of technologies which are gentler on flour structure than heat treatment but effective against common pathogens associated with wheat and raw flour has yet to find that elusive 'silver bullet'.

At this symposium, attendees will learn about the collaboration between a flour producer, a CPG company, and FDA that put in motion a new comprehensive end-to-end approach to reducing the microbiological risks associated with raw flour frozen pizza products. This unconventional team took a risk-based approach that emphasized transparency, sparked some head-scratching, and a bit of ole fashioned discomfort. Presenters will show that solutions do not lie in siloed work streams along the value chain but rather in unique strategies and partnerships where uncomfortable 'gives' result in important 'gets'.

What did we get? A respectful and open relationship based on aggressive risk-management important to public health, new insight into the microbiological quality of flour, a novel application in flour production to reduce pathogen presence with potential to lead to even more discovery, a comfort level with regular testing for release of an agricultural commodity for pathogens of concern, improvement to zoning and separation in a pizza manufacturing process, and a measurably safer process and product that consumers can count on.

S64 Cultivating Meaty Cells – A Perspective Focus on Food Safety, Regulatory, and Experiences

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ANDREW PANTANO: *Upside Foods, Emeryville, CA, USA*
NATALIE RAINER: *K&L Gates LLP, Chicago, IL, USA*
ANGELA ANANDAPPA: *Alliance for Advancing Sanitation and Northeastern University, Glenview, IL, USA*

Global demand for protein continues to accelerate, along with increasing global populations. An growing number of cell-based meat production companies seek to fill the need for protein rich products, while keeping sustainability and nutrition in mind. In 2023, cell-culture meat innovation came to a focal point, with two U.S. companies being awarded FDA/USDA approval (Good Meat and Upside Foods). The competitive landscape is now set for innovation and both start-up and developed companies sit together at the table in this product space. In terms of innovation, cell culture development, scale-up and commercialization leverages less common unit operations (e.g., bioreactors) in elevated, hygienically clean environments. Numerous opportunity areas exist in this space including addressing potential production and development concerns, regulatory approaches, and identifying the current research needs that is being conducted for broader industry education and best practices.

This session will focus on discussing the hazards identified in production chains and developmental stages, shed light on potential production and regulatory pinch points, provide a regulatory perspective of what is being observed and define where current research may and should lead towards.

Target audience members include those interested in cell culture meat as an up-and-coming product category, sanitation professionals looking for education on cleaning and sanitation processes, food safety professionals looking to adequately partner with their innovation and commercialization teams with proper education, and the broader IAFP Annual Meeting attendee pool interested in this relatively unknown product and process set.

S65 Empowering the Detection and Characterization of Foodborne Pathogens Using Artificial Intelligence and Advanced Analytical Techniques

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KENTO KOYAMA: *Hokkaido University, Sapporo, Japan, Japan*
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Foodborne illness investigations trace the cause of the disease to specific foods, allowing public health officials, regulatory agencies, and the food industry to pinpoint probable sources of contamination. However, the intricate nature of food matrices and the diversity of microbiota compositions pose significant challenges in the rapid detection of foodborne pathogens and accurate characterization of how these pathogens respond to the food environment. In contrast to traditional culture-based methods, advanced analytical techniques such as spectroscopy, microscopy, and genomics enable the rapid acquisition of extensive microbial phenotypic and genotypic information. This elevates the accuracy and sensitivity of detection, classification, and characterization. The datasets generated through these advanced analytical techniques contain numerous variables, such as thousands of genes in a bacterial genome and a multitude of pixels in microscopy images, which require comprehensive data analysis. Artificial intelligence (AI), especially machine learning, plays an important role in extracting useful information from data. Through the integration of AI and advanced analytical techniques, we can significantly reduce the pathogen detection time from several days to a few hours with desirable sensitivity. In addition, AI-based approaches can discover new knowledge of microbial responses to the environment and intervention methods. This symposium will focus on the application of AI in microbial detection and characterization. The discussion will provide insights into new factors that are related to pathogen growth and survival, which may impact the rate of pathogenic infections and modulate outbreaks. The technology and knowledge obtained from this symposium will empower the food industries in improving sanitation and control strategies, while concurrently reducing labor and cost overheads.

S66 Training Low-Literacy Groups across Cultures: Balancing Universal Principles and Custom Approaches

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CLARE NARROD: *JIFSAN; U of Maryland, College Park, MD, USA*
JESSIE VIPHAM: *Kansas State University, Manhattan, KS, USA*

Food safety training for food handlers at the farm or in retail settings is often a foundational intervention to stimulate behavior change and capacity building. Whether by itself or as part of a broader approach, training can increase knowledge and motivation, and promote habit formation. Training low-literacy groups, which often face other socio-economic constraints, poses unique challenges which can impact sustained behavior change. This symposium will present case studies of food safety training involving food handlers in Nigeria, Latin America, and Cambodia, in communities characterized by low literacy or limited access to knowledge resources. It will highlight sucessful training approaches and their impact across cultures.

Attendees will gain practical understanding on how to design training strategies that can best support behavior change for low-literacy communities, including:

- What information is essential to design effective training for low-literacy participants
- Which training models have been most successful with those groups
- How training impact can be measured

S67 Unraveling Pathogen Dynamics: Insights from a Multi-Year Collaborative Longitudinal Study in the Southwest

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JULIE ANN KASE: *U.S. Food and Drug Administration, College Park, MD, USA*
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SUSAN LEONARD: *U.S. Food and Drug Administration, Laurel, MD, USA*

Since 2019, the FDA, in collaboration with the University of Arizona, the Wellton-Mohawk Irrigation and Drainage District, local growers and industry groups, has undertaken a multi-year study. This region is a significant hub for the cultivation of leafy greens sold in the United States. The collaboration was initiated following the 2018 *E. coli* O157:H7 outbreak associated with Romaine lettuce. The primary focus of this unique study was to identify the key factors that contribute to the introduction, persistence, growth, and dissemination of pathogens that could potentially contaminate produce. Additionally, it seeks to determine industry best management practices that aid in the reduction of pathogens in the environment and fresh produce.

During this session, we provide an overview of the extensive longitudinal study encompassing collection of environmental samples over the past five years, including irrigation water, soil, sediment, air, animal fecal matter, and other sources. We will give special attention to how geography within the study region and the types of agricultural and other land use activities, such as Concentrated Animal Feed Operations (CAFOs), can potentially influence produce production areas.

Presentations will encompass several key areas of interest including the development of cost-effective passive air sampling methods, the influence of wildlife on the study region, the presence and persistence of STEC and *Salmonella* in the environment, as well as the utilization of whole genome sequencing and metagenomics to enhance our comprehension of how pathogens move throughout the region.

Overall, our aim is to provide the audience with a comprehensive understanding of how diverse environmental factors can impact food safety. We will also explore how the efficiency of various methods can contribute to assessing potential risks to fresh produce and ultimately human health. This knowledge, coupled with findings from similar studies, will aid in refining best practices for growers, empowering them to continually enhance the safety of their products.

S68 New Quantitative Risk Assessment Models for *Listeria monocytogenes*: Insights and Applications

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The persistent trend of worldwide listeriosis has informed the need for undertaking further research work to assess the several pathways of contamination from a farm-to-table continuum, yet considering vehicles of transmission other than the traditional ones. Analysis of epidemiological data between 2010 and 2021 pointed out produce as the second most important known source of listeriosis outbreaks in the USA (after dairy); whereas EU surveillance data have shown that seafood commodities are as important sources of strong-evidence outbreaks as meat products. In response to a request by the Codex Committee on Food Hygiene (CCFH) at its fifty-second session, formal risk assessment models were developed by the Joint FAO/WHO Expert meeting on microbiological risk assessment of *Listeria monocytogenes* in foods; Part 1 (FAO HQ, Rome, Italy: 24 – 28 October 2022), taking into account the effects of agrifood practices, climate change and the latent possibility of cross-contamination along the production chain for produce and seafood commodities. Quantitative risk assessment (QRA) models were subsequently programmed in open-source software, tested and revised at the Joint FAO/WHO Expert Meeting on microbiological risk assessment of *Listeria monocytogenes* in foods. Part 2: Risk Assessment Models (WHO HQ, Geneva, Switzerland: 29 May – 2 June 2023). Thus, the objective of the symposium is threefold: (1) to present an overview of the current knowledge on *L. monocytogenes* in different commodities as well as an update of the hazard characterization models; (ii) to describe the new QRA models for frozen vegetables, cantaloupe and RTE seafood developed by WHO in the light of new data, approaches and methodologies; and, (iii) using the newly developed user-friendly R shiny applications, to demonstrate the exploitability of such models through assumptions and scenarios customisable to low-, middle- and high-income countries.

S69 From Process to Product: Bio-Mapping and Potential Solutions for Ensuring Poultry Product Safety and Sustainability

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HARSHAVARDHAN THIPPAREDDI: *University of Georgia, Athens, GA, USA*
MANPREET SINGH: *University of Georgia, Athens, GA, USA*
JASMINE KATARIA: *Kerry, Beloit, WI, USA*

Bio-mapping studies play a pivotal role as they allows processors to observe process trends, evaluate the effects of process alterations, conduct root cause analyses of events, comprehend the significance of process steps in reducing microbial load, and compile performance data. Peer-reviewed data mining of poultry processing bio-mapping for foodborne pathogens such as *Campylobacter*, *Salmonella* spp. and indicator organisms contributes to informed decision-making for food safety management and manage microbial quality of food and minimize food waste.

Poultry meat can experience temperature fluctuations during refrigerated storage and or abuse, leading to spoilage of the product due to microbial growth. This microbial growth can result in odor, taste, and texture changes, rendering it unsuitable for human consumption and contributing to food waste. Understanding microbial community profiles and identifying spoilage flora could offer valuable insights to develop potential solutions to improve the quality of poultry meat.

Symposium attendees will learn about:

1. The current supply chain and pian points of *Salmonella*
2. The current tools employed to determine microbial contamination levels in processing facilities throughout the production process.
3. Potential solutions for addressing spoilage microorganisms in the final product and reducing food waste.
4. Available tools, industry perceptions, regulations, and antimicrobial interventions to showcase the data and solutions for post-processing up to the final product.
5. Insights into the critical role of data in making food safety decisions within the plant, as well as providing solutions for the final product. Importance of Quantitative Microbial Risk Assessment and Bio-mapping for making safety decisions and allocating priorities.

This session will empower stakeholders in poultry industry with the knowledge and strategies needed to navigate the evolving use of modeling science along with microbiome technology and the importance of biomapping bacteria to foster safer, healthier choices for poultry industry practices and intervention technologies.

S70 Metagenomic Tools for Identifying Eukaryotes and Associated Microbiota in Complex Samples: Challenges and Strategies

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JESSE MILLER: *Neogen, Lansing, MI, USA*

Culture-Independent Next Generation Sequencing (NGS), also known as metagenomics, provides the capability to directly identify the entire community of microorganisms in a food sample. Targeted and target-independent metagenomics have been employed to delineate microbial communities in food and their impact on our gut microbiome, which is relevant to food safety topics such as nutrition and allergies. The accessibility and affordability of NGS technologies, improved bioinformatic pipelines, and converging reference databases further increase the uptake of this technology. Incorporating eukaryotic organisms in metagenomic analyses will enable a more comprehensive taxonomic profiling of the genomic material within a particular food, increasing our understanding of their ecological function and diverse array with microbial interactions.

As the largest and most diverse group of eukaryotic organisms, insects are major competitors for human food sources. Some are pests to agricultural crops and may remain in harvested foods because they cannot be completely eliminated. These are considered non-harmful to humans when present in food up to certain levels. Others adulterate processed food and stored products or have physiology and behaviors that makes them exceptionally capable of spreading foodborne pathogens, potentially representing a risk to consumers. Therefore, gathering comprehensive information on the whole community of organisms that may be present in a food sample is important not only for regulatory purposes, but also for increasing our understanding of the intricate prokaryotic-eukaryotic interactions taking place in a specific food product.

This symposium will integrate metagenomics with target enrichment via hybridization-based capture to efficiently detect insects in food samples. We are expanding the application of metagenomics to detect pathogens in insects sold as feed and food, as well as eukaryotic protozoan parasites in irrigation water samples. This symposium will discuss the potential uses of metagenomics and highlight the full spectrum of genomic tools available for feed and food safety.

S71 Microplastics and Nanoplastics: Are They Really Long-Overlooked Food Safety Threats?

JUSTIN M. GORHAM: *National Institute of Standards and Technology, Gaithersburg, MD, USA*
SARA BENEDÉ: *Spanish National Research Council, Madrid, VA, Spain, Spain*
SONIA SU: *Cornell University, Ithaca, NY, USA*
STACEY WIGGINS: *U.S. FDA, College Park, MD, USA*

Microplastics (< 5 mm in their longest dimension) and nanoplastics (< 100 nm) are garnering increasing attention from academia, industries, regulatory agencies, and the general public. Recent studies have shown that those microscopic particles migrate from environmental sources, such as soil, or common plastic food contact surfaces, such as plastic food packaging, into our food. Micro- and nanoparticles (MNP) have been found in sea salt, honey, fruit, shellfish, beer, bottled water, among others. Nanoplastics are of particular concern as they can more easily penetrate biological barriers and enter vasculature, consequently reaching and accumulating in various organs – including human placenta. While our understanding of the toxicity of MNP, especially that in the long term, is still evolving, there is an urgent need to determine the prevalence and abundance of MNP in food. This knowledge will prove critical to the establishment of regulation and the development of mitigation strategies for MNP in food.

In this symposium, we feature the latest progress on understanding the sources of MNP and their occurrence in food, methods for recovering and characterizing MNP, current regulatory landscape, and potential mitigation strategies.

Roundtable Abstracts

SS1 Third Get-Connected Market: Connecting More IAFP Professionals on Food Safety in Africa!

SONJA JONES: *USDA-FSIS Atlanta District, Locust Grove, GA, USA*
MATTHEW STASIEWICZ: *University of Illinois, Urbana, IL, USA*
CATHERINE BESSY: *FAO, Rome, Italy, Italy*
AUGUSTINE OKORUWA: *GAIN – Global Alliance for Improved Nutrition, Abuja, Nigeria, Nigeria*
ELISABETTA LAMBERTINI: *Global Alliance for Improved Nutrition (GAIN), Washington, DC, USA*
HUNG NGUYEN-VIET: *International Livestock Research Institute, Nairobi, Kenya, Kenya*
ROBERT FERGUSON: *Food Safety Magazine, State College, PA, USA*
IZANNE SUSAN HUMAN: *Cape Peninsula University of Technology, Cape Town, Western Cape, South Africa, South Africa*
ROSE OMARI: *Science and Technology Policy Research Institute, Council for Scientific and Industrial Research (CSIR-STEPRI), Cantonments, Ghana, Ghana*
TITILAYO FALADE: *International Institute of Tropical Agriculture, Ibadan, Nigeria, Nigeria*
KEBEDE AMENU: *College of Veterinary Medicine and Agriculture, Addis Ababa, Ethiopia, Ethiopia*
ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture, Abeokuta, Abeokuta, Ogun State, Nigeria, Nigeria*

Note: This is not a RT, but an interactive "special session.

The African continent is enormously large, with 55 countries that are very diverse in social, cultural and economic aspects. For several years, food safety professionals from the African continent have been increasing their links with and presence in the IAFP community. There now is a first IAFP chapter in Africa, the African Continental Association for Food Protection.

At IAFP 2022, the proposers first organized this innovative "special session" that aimed to better connect IAFP attendees from Africa with those from around the globe on the challenges to Food Safety on the African continent. It was held again at IAFP 2023, but due to visa issues many of the proposers and potential participants from Africa and elsewhere, could not attend. Therefore, a third run of the "special session" is proposed for IAFP 2024.

The objective of connecting food safety professionals in the IAFP community with an interest in food safety in Africa is to help establish networks and to share ideas and experience that possible will develop further into e.g., collaborations, projects, training and knowledge sharing activities that help further advance food safety in Africa.

The innovative special session format allows for a very active interactions among IAFP professionals from Africa and the rest of the world. The layout of the meeting room allows "marketeers" to display their ideas or wishes on advancing food safety in Africa in order to attract "browsers." A small number of marketeers kicks off the session with elevator-pitch presentations.

There was quite a buzz and a lot of energy amongst the participants in Pittsburgh and Toronto. The proposers (= the organizers and panellists) believe a larger audience of "marketeers" and "browsers" can be attracted in Long Beach. The new format has been shown to work and there is ample opportunity to advertise it more and earlier to the IAFP community.

RT1 The Inclusion of Foreign Material Inspection and Foreign Material Forensics in Food Safety Programs and Management

NICOLETTE BROWN: *Hills Pet Nutrition, Kansas City, MO, USA*
DANIELLE RICHARDSON: *ConAgra, Omaha, NE, USA*
ROBIN FORGEY: *Costco Wholesale, Issaquah, WA, USA*
GALE BEARD: *Grande Cheese, Fond Du Lac, WI, USA*
JOSEPH HOLT: *OSI Group, Aurora, IL, USA*
APRIL BISHOP: *TreeHouse Foods, Oak Brook, IL, USA*

As illustrated by the recent development of the Physical Hazards and Foreign Material PDG, Foreign Material Contamination (Physical Hazards) is a growing concern in many areas of the supply chain, from raw materials through retail / restaurant use. As such it is important to consider and discuss options and considerations for all types of inspection, including X-Ray Inspection and Forensics that can help manufacturers keep product in commerce and reduce food waste.

This roundtable will explore handling episodic events, establishing proactive processes and systems in manufacturing and retail / food service and how handling the events can not only reduce waste but help companies meet sustainability goals and customer requirements, both domestically and globally, while helping team members include Foreign Material Contamination discussions as a part of their Food Safety Culture and Development processes.

Panelists, which will represent multiple food disciplines including retail / food service, processed foods, protein and pet food, will be asked to expand on real life examples, program development, future needs and inclusion of Foreign Material Inspection into their Food Safety Programs and Management. These discussions will directly address Non-Microbiology Food Safety Issues, General-Food Protection for the Future, Developing Food Safety Professionals and Developments in Food Safety Education which are identified as key goals through IAFP. Examples of key discussion topics will include, but not be limited to:

- Handling unexpected events
- How adding Forensic Analysis to contaminant recovery can further enhance programs and corrective actions.
- Risk Management / Assessment considerations as part of Foreign Material Inspection Programs
- Meeting supply chain requirements
- Developing and implementation of possible routine programs and what might be considered for those programs.
- Best practice considerations when handling an event.
- Impact of product salvage on sustainability goals
- Inclusion of Foreign Material Processes in employee training / development tools and practices as we work to continuously development food safety professionals.

RT2 Detection of Enteric Viruses, Methodological Considerations and Interpretation of Results: Scientific Findings of an Expert Panel

JAN VINJÉ: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
BRANKO VELEBIT: *Institute of Meat Hygiene and Technology, Belgrade, Serbia, Serbia*
ALBERT BOSCH: *University of Barcelona, Barcelona, Spain, Spain*
NEDA NASHERI: *Health Canada, Ottawa, ON, Canada, Canada*
LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*

Over the last decade, many national entities (including Canada, various European Union states, the United States, and New Zealand, among others) have completed surveillance programs, the purpose of which has been to evaluate fresh and frozen berries for evidence of contamination with enteric virus (human norovirus and hepatis A virus). The standard methods for detection of viral contamination in foods relies on concentration and purification of the pathogens from the food matrix, followed by detection using RT-qPCR, with or without further characterization using genomic sequencing. Despite validation, these methods are not without ongoing challenges, especially since the detection of viral RNA cannot be unequivocally correlated with the presence of infectious virus in the sample.

In response to these challenges, in 2019 the American Frozen Foods Institute (AFFI) convened an international panel of scientists with expertise in food virology, with specific focus on detection methods. Their deliberations were delayed with the SARS CoV-2 pandemic, but they recently completed a consensus document. Major findings of the expert panel can be summarized as follows: (i) while the ISO 15216 method prevails, there are country-by-country differences in methodology; (ii) sampling plans are diverse, sample sizes are small (25–50 g of fruit) and amplification volumes, smaller; (iii) there is great diversity in interpretive criteria for RT-qPCR results; (iv) confirmatory (sequencing) methods are sporadically used; (v) regulatory actions based on results vary by country/results/product; and (vi) the relationship between viral RNA detection, particularly at high Ct, and public health risk, is unknown. The panel's conclusions will find relevance not only in the way surveillance data is analyzed and interpreted, but also to inform how the globally diverse supply chain will contend with routine monitoring of frozen berries.

In this roundtable (RT), a subset of the experts will convene to discuss the findings of their deliberations. The RT will begin with a 30 min synopsis of key results, with scientific justification, followed by an interactive hour in which experts can engage with the audience in a lively discussion on where the methodology currently stands, and priorities for future refinements.

RT3 Unraveling the Secrets of Sanitation Programs: How to be a Sanitation Change Agent for Decontamination in Retail Food Establishments

GLENDA LEWIS: *U.S. Food and Drug Administration, Washington, DC, USA*
DUSTIN METZGER: *Kwik Trip Inc., LaCrosse, WI, USA*
EMILY CRISPELL: *Chick-fil-A, Inc., Dunwoody, GA, USA*
ANGELA FRASER: *Clemson University, Clemson, SC, USA*
CHARLES PETTIGREW: *Arxada, Morristown, NJ, USA*

Cleaning and sanitizing are not alluring tasks but neither is the 57% of establishments the FDA's 2023 Retail Food Risk Factor study found to be out-of-compliance for this category. This area remains an unmet need for the industry. Unlike sanitizers and disinfectants, cleaners and tools lack a regulatory body that outlines quantitative soil removal standards. Thus, sanitation practitioners are left to their own senses for determining cleanliness under the "clean to sight and touch" rule. Consequently, food safety leaders and buyers must choose among a myriad of cleaning products with widely diverging efficacies and design targets. Unfortunately, inadequate choices of cleaning products and tools or shortcutting cleaning procedures result in unperceivable, lingering food soils that obstruct disinfectants or sanitizers from providing their full efficacy. Overtime these choices balloon into a sanitation program nightmare, e.g., large product lists, expired products, different dilution tips for the same product, off-label usage, shortcuts, safety issues, etc., which inevitably increase food safety risk. In this context, our diverse panel, composed of members from industry, academia, and government will share their successes and failures while also highlighting best practices for adopting new systems and products within an organization. They will also cut through the marketing and demystify chemicals to help end users make the right chemical and tool selection. Roundtable attendees will leave with a better understanding of how to navigate the ambiguous, immense universe of cleaning strategies in retail foodservice establishments so they may maximize their chances of meeting sanitation goals required by their regulatory authority.

RT4 Beyond Root Cause: Targeting Pathogen-Food Pairings and Tailoring Strategies to Prevent Future Foodborne Illnesses

STEPHANIE SMITH: *Washington State University, Pullman, WA, USA*
DONNA GARREN: *American Frozen Food Institute, Woodbridge, VA, USA*
DE ANN DAVIS: *Western Growers Association, Pacific Grove, CA, USA*
MICHELLE KUSNIER: *Michigan Department of Agriculture and Rural Development, Flint, MI, USA*
KRISTINE GASPERIC: *Indiana Department of Health, Indianapolis, IN, USA*
MARK MOORMAN: *FDA, Washington, DC, USA*

Foodborne illness outbreak investigations and root cause analyses have identified patterns of pairings between certain disease-causing microorganisms with specific food commodity types. Identifying these patterns and determining the underlying reasons why and how the food contamination happens are foundational to stopping them from repeating. But to truly break these patterns of reoccurring outbreaks requires tailored prevention strategies. The best strategies are crafted from robust stakeholder input, open and honest communication, considerate of mechanisms to measure progress and translatable to clear courses of action by producers and consumers.

This session features examples of forward-thinking prevention strategies and innovative approaches from industry, regulatory, and academia tackling the biggest challenges to food safety, including:

- Data-driven design and stakeholder engagement to create national prevention strategies for soft fresh queso fresco-type cheese, imported enoki and wood ear mushrooms, bulb onions and powdered infant formula;
- Targeted regional surveillance samplings and creating prevention consumer messages to mitigate melons-related *Salmonella* outbreaks;
- California Ag Neighbor program that brings livestock and produce communities together to prevent future outbreaks;
- Artificial intelligence simulated environments to design production facility-specific environmental sampling strategies and dynamic food safety assessment systems; and
- Translating foodborne illness outbreak lessons learned into clear and actionable prevention guidance for small businesses.

We discuss how these prevention strategies are created, communicated, trained on, and disseminated to the public in order to promote behavior change and improve practices that mitigate food safety risks.

RT5 Understanding the Risks of Foodborne Viruses in Foods and Water

JACQUELINA WOODS: *U.S. Food and Drug Administration – Gulf Coast Seafood Laboratory, Dauphin Island, AL, USA*
JEFFREY LEJEUNE: *FAO, Rome, Italy, Italy*
LEE-ANN JAYKUS: *North Carolina State University, Raleigh, NC, USA*
KALMIA KNIEL: *Department of Food Sciences University of Delaware, Delaware, DE, USA*
MARCIANE MAGNANI: *Federal University of Paraiba, João Pessoa, Paraiba, Brazil, Brazil*
JULIE JEAN: *Institut sur la nutrition et les aliments fonctionnels, Université Laval, Québec, QC, Canada, Canada*

Foodborne viruses cause millions of gastroenteritis cases worldwide, resulting in many hospitalizations and deaths every year. Norovirus, hepatitis A, and hepatitis E are among the most important foodborne viruses. Foods can be contaminated through utensils or food contact surfaces during processing or by infected food handlers. Contaminated water used for irrigation, in food processing or as a food ingredient can also lead to contamination. Studies of viruses in foods are challenging in part because of the complexities in culturing viruses outside their animal hosts. Viral surrogates (e.g., non-pathogenic bacteriophages) are used to approximate the characteristics of viral pathogens and can be more easily studied due to lower biosafety levels, and easier propagation and detection techniques. Surrogate data can help to estimate the virus behavior and the antiviral effectiveness of sanitization procedures and food processing technologies. Unfortunately, validated surrogates are not available for all foodborne viruses. Roundtable participants will discuss challenges related to the main foodborne viruses in foods and water. The risks of food contamination in the whole food chain will also be discussed. Panelists will debate the limitations on estimating risks using surrogates. Panelists will also engage with questions and comments from the roundtable attendees.

RT6 Ingredient Safety: Current Perspectives from Food Toxicologists

RANDOLPH DUVERNA: *United States Department of Agriculture, Food Safety and Inspection Service, Washington, DC, USA*
ERIC SCHWARTZ: *Food Chemicals Codex, Rockville, MD, USA*
KRISTI MULDOON JACOBS: *U.S. Food and Drug Administration, Rockville, MD, USA*
ELISABETH ANDERSON: *Michigan State University, Lansing, MI, USA*
RENE VINAS: *UPSIDE Foods, Atlanta, GA, USA*
PAUL HANLON: *Abbott Nutrition, Columbus, OH, USA*

As consumer preferences and demands continue to evolve, food manufacturers are continually exploring innovative ingredients to enhance product offerings, and this evolution is occurring in parallel with processes that are always running to ensure the safety of food products. In this roundtable session, panelists from industry, academia, and government will provide their perspectives on processes used to ensure the safety of food ingredients in regard to food chemicals. Attendees will gain insights into the challenges faced by regulatory bodies and industry stakeholders in evaluating food ingredients in two main areas: processes for approving novel ingredients, and the importance of establishing specifications for food ingredients. The discussion will delve into the complexities surrounding novel ingredient approval, including risk assessment, toxicological considerations, and potential allergenicity. Subsequently, the session will delve into the critical role of establishing and implementing precise ingredient specifications. Participants will gain an understanding of how accurate specifications aid in guaranteeing consistency and quality, enabling manufacturers to meet regulatory standards and exceed consumer expectations. The audience is encouraged to participate in the session with questions they have for food toxicologists. The proposed session aspires to foster meaningful discussions and knowledge exchange among food safety professionals, regulators, and industry stakeholders, driving the IAFP objective of continuous improvements in ingredient safety and safeguarding the health and trust of consumers worldwide.

RT7 Establishing Clean Breaks – Hygienic Separation of Production in Low-Water Activity Foods

MISTELLE SIGNOR: *Mennel Milling, Fostoria, OH, USA*
JEREMY TRAVIS: *Hilmar Cheese & Ingredients, Hilmar, CA, USA*
DANIEL BELINA: *Land O'Lakes, Saint Paul, MN, USA*
JOHN HOLAH: *Kersia Group, Bury, United Kingdom, United Kingdom*
DAVID CLIFFORD: *Nestlé USA, Inc., Solon, OH, USA*
BENJAMIN WARREN: *FDA-CFSAN, College Park, MD, USA*

Conversations surrounding the establishment of clean breaks without the use of a wet-wash are commonly encountered and can be controversial. In many operations water is avoided as much as possible because it can actually cause the opposite of the desired effect by allowing dormant microorganisms to grow rapidly. Numerous factors can influence the definition of a 'lot' in these operations and this can have major implications, especially in long-run/continuous operations. Regardless of how it is defined by a producer, lot definition is a critically important decision for traceability purposes and to mitigate risks in the event of a microbial contamination incident.

The challenge of maintaining and implementing sanitation for the purpose of lot definition in the production of low-water activity foods such as infant formula, dry milk powders, flour, peanut butter, chocolate and other matrices introduces an added layer of complexity to the discussion on clean breaks. The use of water and potential introduction into these products can facilitate microbial growth; hence, alternative dry sanitation or novel techniques must be employed to ensure a clean break while maintaining product quality and safety. To address the challenges of establishing clean breaks, including overcoming the cultural and learned behaviors already existing within a facility, to isolate production lots in low-water activity foods, this roundtable will explore various approaches to defining clean breaks across different industries. This discussion will encompass existing resources, as well as innovative solutions to this issue and their regulatory implications. Furthermore, the panel will delve into the use of a risk analysis approach to strike a balance between the food safety mindset and the business mindset.

RT8 Laboratory and Regulatory Challenges of Probiotics in Food Products

MARK SKASKO: *FDA, Division of Animal Food Ingredients, Rockville, MD, USA*
MICHELE SAYLES: *Diamond Pet Food, Topeka, KS, USA*
DANA BUCKMAN: *Bioform Solutions, San Diego, CA, USA*
MARIE-EVE BOYTE: *NPCS International, Greater Montreal, QC, Canada, Canada*
JEAN SCHOENI: *Eurofins Microbiology Laboratories, Inc., Madison, WI, USA*

In the evolving landscape of food science and health, the integration of probiotics into both human and animal diets has gained significant traction. Probiotics have transitioned from being primarily supplements to being incorporated into a wide array of food products, including infant formula, dairy items, cereals, snacks, beverages, pet foods, and treats. This integration of probiotics necessitates a comprehensive understanding of how to ensure the safety and efficacy of these products. These products often make various health claims, besides effects of biopreservatives in many, such as enhancing gastrointestinal health, aiding weight loss, boosting immunity, and even improving memory. Regardless of the claimed effects, the labeling of live organism counts in these products poses a challenge for validation and verification pertaining to regulatory compliance, impacting food safety and integrity.

This roundtable proposal seeks to facilitate a constructive dialogue among researchers, food manufacturers, regulators, and related industries. The discussion aims to contribute to the advancement of food safety protection demands and meeting the regulatory requirements within this evolving domain of probiotic-enhanced food items. The panel will address the challenges in establishing testing methods, validating label claims, and navigating regulatory and labeling requirements within the context of probiotics in food products.

Key Objectives of the Roundtable Discussion:

1. Historical Perspective on Probiotics in Food Products:
   A brief overview of the historical use of probiotics as supplements and their transition into various food products, and the diverse claims made and typical labeling requirements of these food products, such as label guarantees and CFU/g counts.
2. Probiotic Selection and Testing Methods:
   An exploration of the most commonly used probiotics in the food industry and an examination of the available methods for enumerating and verifying probiotic content in food products. Experts will discuss the advantages and limitations of different methods.
3. Challenges in Probiotic Recovery:
   Participants will engage in discussions about the complexities and challenges of recovering probiotics in different food products. This topic will address issues related to viability, stability, and efficacy in real-world applications.
4. Regulations, Compliance, and Labeling:
   Regulatory experts will provide insights into the intricate landscape of labeling and compliance for food products containing probiotics. This session will offer advanced information on the legal requirements and standards governing probiotic product labeling.

By fostering collaboration, promoting innovation, and prioritizing consumer well-being, this Roundtable aims to shed light on laboratory and regulatory issues related to the integration of probiotics into food products. Professionals from various sectors are invited to join this transformative discussion, addressing critical aspects of probiotics in the context of food protection.

RT9 Don't Let It Happen Again! Avoiding Another GIANT Recall in Aseptically Packaged Foods and Beverages

ROBERT W. MANNING: *Liquid Consulting, Sanford, FL, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
YUQIAN LOU: *PepsiCo, Purchase, NY, USA*
ROBYN EIJLANDER: *NIZO Food Research, Ede, Netherlands, Netherlands*
ANDRÉ REHKOPF: *Saputo, Sacramento, CA, USA*
CORPUS PEREZ: *Reckitt, Evansville, IL, USA*

Aseptically processed and packaged products enjoy a remarkable history of microbiological food safety and stability. However, a major voluntary recall was initiated in the latter part of 2022, impacting tens of millions of containers across dozens of products of multiple brands. As a result, the FDA conducted an inspection of the affected processing facility and issued a warning letter, dated January 30, 2023, detailing numerous observations and potential deficiencies in the operation. The information in the warning letter provides useful insights into what may go wrong in an aseptic processing and packaging operation, and presents the opportunity to develop strategies to prevent this type of event from happening in the future.

While still relative rare, commercial losses and recalls due to the spoilage of aseptically packaged shelf-stable and extended-shelf-life (ESL) refrigerated products are increasing in frequency due to a variety of reasons. These include new and inexperienced entries to the market segment, longer production runs, use of new and exotic plant-based ingredients, difficulty in hydrating hydrophobic ingredients, and high bacterial spore loads in the incoming ingredients. Once a spoilage event occurs in this category, the root cause of spoilage is exceedingly difficult to diagnose due to the size and complexity of the processing and packaging lines, the length of production runs, and understanding the significance of the type of microorganisms found in the affected product. These challenges call for holistic and multidisciplinary contributions to provide for root cause-analysis and prevention of spoilage issues.

The objective of this roundtable is to provide insight into procedures and strategies to investigate and prevent spoilage issues in this category. Panelists, including FDA representative(s), will use their expertise and practical experiences to examine the information detailed in the FDA warning letter and interpret its content into practical strategies to prevent spoilage and recalls in this product category.

RT10 Think Like a Criminal – The Dark World of Food Fraud

DELEO DE LEONARDIS: *Purity-IQ, Guelph, ON, Canada, Canada*
CLARE WINKEL: *Integrity Compliance Solutions, Brisbane, Australia , Australia*
JESSICA BURKE: *BRCGS, Milton, ON, Canada, Canada*
ALLISON JORGENS: *Loblaw, Toronto, ON, Canada, Canada*

Food fraud occurs when a food product is intentionally misrepresented or adulterated for the purpose of financial gain. Activities include substitution, dilution, mislabeling or through making false claims. It is estimated that food fraud affects roughly 10% of all commercially sold food products most often reported in olive oil, honey, spices, fish, juices and organic products, costing governments alone billions annually.

Products may be impacted from a quality perspective however the impact to food safety is a significant concern, including recent increased risk due to supply chain challenges. According to the OPSON IX report, recent disruptions to the supply chain enabled opportunistic criminals to take advantage of new vulnerabilities. In 2023, Lithuanian authorities identified a group chemically altering best before dates on expired meat products, resulting in one million food products being seized from 70 locations with 24 arrests.

During 2015 a significant recall occurred linked to cumin adulterated with ground peanut and more recently in 2023, Italian authorities investigated a case of food fraud in fish, where products had been defrosted and adulterated with large amounts of undeclared nitrites and nitrates to extend their shelf life and improve their "fresh" appearance, this resulted in the hospitalization of 27 people and the arrest of 11 suspects.

Despite the ongoing work of government agencies, industry and academia working diligently to detect and deter food fraud activities issues continue to occur potentially impactful to human health.

Regardless of the all the work and oversight many food producers and retailers remain "in the dark" when it comes to understanding what is required to effectively manage this food safety risk. This roundtable will invite the audience to "think like a criminal" when assessing their supply chain for potential food fraud risks. The panel, made up from industry, certification, retail and laboratory experts, will discuss best practices and strategies for mitigating the risk of food fraud and shining light into these murky activities.

RT11 *Cronobacter* spp. Control: Bridging Knowledge Gaps and Taking Action

JOHN HANLIN: *Ecolab Inc., Eagan, MN, USA*
JACK VAN DER SANDEN: *BioMerieux, Durham, NC, USA*
RANDY PORTER: *Institute for Environmental Health, Summerville, SC, USA*
JEFFREY FARBER: *JM Farber Global Food Safety Consulting, Thornhill, ON, Canada, Canada*
DAVID CLIFFORD: *Nestlé USA, Inc., Solon, OH, USA*
BENJAMIN WARREN: *FDA-CFSAN, College Park, MD, USA*

Powdered infant formula (PIF) is a nonsterile food product that may be consumed by neonates and other infants vulnerable to foodborne pathogens. During manufacturing of PIF and other low moisture foods, the control of environmental pathogens, such as *Cronobacter* spp. and *Salmonella*, is crucial. *Cronobacter* spp. is a ubiquitous bacterium found naturally in the environment and is particularly adapted to surviving in low moisture foods, such as PIF and powdered milk used for making PIF, and is, thus, a pathogen that must be controlled during production of these commodities. *Cronobacter* spp. can contaminate the product in various stages of the processing from raw materials, during production, or at home through contaminated water and feeding items. One of the main control measures for *Cronobacter* spp. during manufacturing is minimizing or limiting water in the dry production areas; however, controlled sanitation or cleaning may be necessary at some intervals. Moreover, mitigation of *Cronobacter* spp. in a contamination event while minimizing water is challenging. Additionally, knowledge gaps still exist on epidemiology, prevalence, and effective mitigation in dry environments for *Cronobacter* spp. The recent passing of *Cronobacter* spp. as a nationally notifiable disease may increase detection and whole genome sequencing of *Cronobacter* spp. and help fill existing knowledge gaps. In this roundtable, regulators, researchers, and manufacturers will discuss recent findings on the management and control of *Cronobacter* spp. in low-moisture production environments.

RT12 Code Club: Leveraging Statistical Programming to Get the Most from Your Data

PRAMEY KABRA: *Purdue University, West Lafayette, IN, USA*
KAITLYN CASULLI: *University of Georgia, Athens, GA, USA*
RIGO SOLER: *Texas Tech University, Lubbock, TX, USA*
ABHINAV MISHRA: *University of Georgia, Athens, GA, USA*

The food industry witnesses constant growth, thus proper data management for decision making has become a linchpin for success. The capacity to collect, analyze, and efficiently visualize data has become a necessity in the industry. The emergence of open-source statistical software as compared to software packages has transformed the way scientists and researchers conduct their statistical analyses. The exponential growth in the amount of data that the industry produces has created the necessity for robust software tools capable of illuminating patterns, trends, and insights in this sea of data.

Numerous statistical programming languages could be used in the industry, with one of the most versatile being R and others being Matlab, iPMP, SAS, iRisk, and JMP, especially notable for its capabilities in the field of visualization. The food industry encompasses multifaceted processes, including supply chain management, product development, quality assurance, and market analysis – all of which stand to benefit from the power of data visualization. In an era where informed choices can make or break a business, the utilization of statistical software, emerges as a potent tool for effectively navigating the complexities of the modern food industry. Unlike the usage of commercial software which requires less usage of coding, R requires prior knowledge of the methods to be used and their application methods. This symposium aims to educate students and users from different backgrounds how these tools and packages could be sued for meaningful interpretation of data.

Symposium attendees will learn:

1. Computational statistics programs that can facilitate more efficient data processing and interpretation.
2. Cross-functional collaboration and learning between "wet" and "dry" lab methods to develop them as holistic scientists.
3. Data analyzing methods to understand the usage of tools with or without prior experience of coding knowledge.
4. Overview of popular recent analysis packages for use in microbial modelling and microbiome analysis.

RT13 Can Food Manufacturers Afford Not to Use Whole Genome Sequencing?

BRAD BROWN: *U.S. Food and Drug Administration, College Park, MD, USA*
DOUGLAS MARSHALL: *Eurofins, Fort Collins, CO, USA*
DEANN AKINS-LEWENTHAL: *Mondelez, Omaha, NE, USA*
LESLIE HINTZ: *U.S. Food and Drug Administration, College Park, MD, USA*
SHAWN STEVENS: *Food Industry Counsel, LLC, Milwaukee, WI, USA*
BILL MARLER: *Marler Clark, The Food Safety Law Firm, Seattle, WA, USA*

Environmental pathogens (such as *Listeria monocytogenes*, *Salmonella* spp., and *Cronobacter* spp.) continue to contaminate ready-to-eat foods, resulting in costly product recalls and foodborne outbreaks. Food manufacturers typically implement environmental monitoring programs and product testing programs for these pathogens, as appropriate to their products and processes. When an environmental pathogen is detected by a food manufacturer, corrective actions must, among other things, identify and correct the problem and reduce the likelihood that the problem will recur. Analyzing and investigating pathogen isolates using whole genome sequencing (WGS) can provide the most complete information available

to identify and implement appropriate and effective corrective actions. Used in this way, WGS can be a powerful tool for resolving contamination issues and preventing foodborne illnesses and outbreaks, as well as the associated costs. Yet despite this opportunity, many food manufacturers are reluctant to incorporate WGS into their corrective actions when they identify a pathogen in their product or process. The cost of the analysis itself as well as the liability of identifying persistent contamination within their facility (e.g., a resident strain) and/or a connection between environmental isolates and clinical isolates are often cited by food manufacturers as reasons for not incorporating WGS into their corrective actions. Essentially, food manufacturers have felt they could not afford the liability of generating WGS results and the that comes with it. However, there are many examples of foodborne outbreaks where the investigation revealed that the responsible food manufacturer had identified the causative pathogen in their product or process before the onset of the outbreak, meaning that the timely use of WGS and appropriate actions by the food manufacturer could have significantly reduced the number of people harmed by the contaminated product, or prevented the outbreak altogether. More recently, the cost-benefit for food manufacturers using WGS has been demonstrated (outside of potential civil or criminal liability). The question now becomes…can food manufacturers continue to afford not using WGS?

RT14 Importance of Outreach to Spanish-Speaking Growers and Farmworkers to Ensure Food Safety for U.S. Consumers

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U.S. imports have increase by 200% over the past two decades, receiving fresh produce from more than 125 different countries, with Mexico producing 77% of those imports. Domestically, 70% of agricultural workers in the U.S. speak a foreign language, mainly Spanish. This illustrates the importance of expanding the education and outreach efforts in Spanish, to make market and regulatory requirements known and achievable for growers and farm workers, including importing farms. It is widely known that effective training and educational resources support agricultural workers as they strive to reduce microbial risks to fresh produce and protect U.S. consumers. Regulatory agencies, non-profit associations, researchers, extension personnel, and members of academia have noted this growing necessity for outreach and extension focused on Spanish-speaking growers and farmworker communities and so have developed different education and outreach strategies. However, we continue to find growers seeking outreach and education attention in their preferred language. This session is intended to serve as a platform to identify innovative outreach strategies and to build collaborations and partnerships to serve U.S. and international Spanish-speaking growers and farmworkers. We will discuss the challenges in finding and reaching these audiences, who are critical players in ensuring food safety for the U.S. consumer.

RT15 How Did FDA Define Strong Evidence for Food Traceability List (FTL) Foods and What are Its Implications for the Future?

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The Food Traceability Rule (FSMA 204) requires increased tracing for certain foods as determined by FDA's Risk-Ranking Model for Food Tracing (RRM-FT). But what, exactly, is the RRM-FT and how do foods and ingredients land on the list? Additionally, with the rule also setting forth a process for the FDA to update the Food Traceability List (FTL) when deemed necessary. When and how might the agency determine a food should be added to – or dropped from – the FTL?

To set the stage, the roundtable will start with a brief presentation of key aspects of the RRM-FT by a member of the FDA model development team. The industry and regulatory experts on the panel of this roundtable session will then discuss the semiquantitative, multicriteria, decision-analysis approach of the RRM-FT; how it aligns with the factors specified in section 204(d)(2) of FSMA; how it scores commodity-hazard pairs (e.g., Shiga toxin-producing E. *coli* O157 in leafy greens); and how a "high" score on the RRM-FT's seven criteria (frequency of outbreaks and illness, severity of illness, likelihood of contamination, potential for pathogen growth considering shelf life, manufacturing process contamination probability and intervention, consumption rate and amount, and cost of illness) can put a food on the FTL.

As outbreak detection improves through the use of advanced molecular methods, new pathogen and commodity pairs may be identified based on a "strong-evidence" threshold (considering severity and likelihood of a hazard), and a commodity put on the FTL even if it scores below 330 in the Model. The panel will discuss characteristics that might cause a commodity to be added or removed from the list, address how FDA can propose updates to the list, and explain how stakeholders can have a say in proposed changes. Participants will leave the session with a better understanding of the RRM-FT Model, and how reducing certain risks may (potentially) help to keep foods off the list.

RT16 Advancing Food Safety Regulation: A Globally Applicable Maturity Model

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The global food safety regulatory landscape has undergone a significant transformation over the past two decades. As we approach 2025, the realm of food safety regulation and related research faces a critical juncture. Current research in this domain often focuses on the implementation of regulations and safety management systems, examining their direct impact on public health outcomes. Concurrently, other studies delve into evaluating cultural, climatic, and behavioural shifts within private food entities, assessing their influence on trade. However, it is imperative for all food safety regulatory authorities (FSRAs) to assess their effectiveness, the capacity for continuous improvement, and their ability to optimize processes.

These assessments are crucial as the impact the quality of services rendered, stakeholder confidence, access to international markets through favorable trade agreements, and global food security. In 2022, Nayak & Jespersen identified globally applicable building blocks of mature FSRAs, laying the foundation for a maturity model discussed during a roundtable session at IAFP 2023. This model aims to assess and benchmark the effectiveness of FSRAs worldwide, facilitating ongoing improvement and optimization.

These building blocks enable regulators to evaluate their growth stage and formulate improvement plans using a self-assessment tool, the maturity model, supported by food safety regulators globally. Over the past year, we've developed a maturity model that considers diverse practices adopted by FSRAs, addressing local and global challenges and mitigation strategies for impacts on public health and trade.

While promoting the shared vision of a globally sustainable, safe food supply, the model offers insights into managing conflicting priorities, such as trade versus public health and changing political dynamics within the FSRA domain. The subsequent roundtable session will include panel members from regulatory agencies and industry worldwide to further explore the foundational building blocks' applicability in navigating the complex factors influencing FSRA operations. This collaborative effort seeks to advance food safety regulation on a global scale, enhancing the safety and security of the global food system.

RT17 Transitioning from Grad School to Professionals: Insights from Recent Graduates

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CARLA SCHWAN: *University of Georgia, Athens, GA, USA*

Embarking on a graduate degree can be a daunting challenge for students, and once graduation approaches, additional stresses arise as students navigate the next steps in their careers. While these changes can be intimidating, gleaning insights from individuals who have effectively transitioned from school to the professional sector can illuminate a path forward and help ease this inevitable phase. Deciding between academia, industry, or government, and whether to pursue a doctorate degree can be overwhelming. Oftentimes, having heard the perspective of professionals who have already gone through that transition can help clear some questions and concerns regarding the best decision for each individual. For international students, the decision to stay in the U.S. or return home adds another layer of complexity to the process.

To help ease this transitional phase, our roundtable discussion will invite professionals who have made the shift from student life to the workforce within the last five years. Our panel will feature experts who have ventured into academia, industry, and government roles, each offering their unique perspectives and sharing their personal experiences to illuminate the path forward for transitioning students. This discussion will benefit students who are close to graduating and thinking about the next steps in their careers, as well as young professionals who recently started a professional career and are struggling with the transition. The discussion points will include the hurdles encountered during this transition and strategies employed to overcome them, as well as the resources and insightful tips that propelled these professionals through their journey, and the invaluable advice they can dispense to those on the cusp of graduation.

RT18 Transitioning from Auditor to Coach: Reimagining Retail Audits to Build Collaborative Relationships and Dissolve the "Us vs. Them" Mentality

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KARLA ACOSTA: *The Acheson Group, San Angelo, TX, USA*
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Evaluating the effectiveness of food safety programs in retail establishment is often limited to intermittent inspections, by internal and/or external entities. Due to the observer effect, the perceived punitive nature of inspections, and a lack of standardization across auditors, the standard inspection as it stands today may not accurately depict the strength – or the lack of – food safety within an establishment. By reimagining inspections and audit practices, food safety culture will be improved, and food safety programs will be strengthened. Inspections are used to assess safe food practices in retail establishments, but often miss the opportunity to coach, teach, and build strong relationships. As inspections inherently involve evaluation of performance, it can be challenging to reinforce good food safety practices while also fostering trusting, working relationships between auditor and auditee.

Through reimagining inspections and audits, a more accurate understanding of food safety practices will unfold from all perspectives. Retail food safety inspections, whether internal or from a regulatory agency, are often ambiguous experiences for retail employees, with follow up only coming after a poor inspection. One of the biggest challenges that comes from inspections is the observer effect where employees may alter their practices for the sake of performing well on an audit, then revert once the audit has concluded, providing an inaccurate reflection of the food safety programs and food safety culture of the establishment.

This session gathers retail industry members and regulatory personnel experienced in internal audits and inspections to explore current practices for conducting audits, opportunities to enhance auditing programs to foster a more mature and inclusive food safety culture, and methods to encourage industry-regulatory collaboration. Panelists will speak to a variety of topics, including:

- Methods for conducting standard food safety inspections that bridge the gap between store auditor and auditee.
- How to take meaningful action based on collected data that strengthen food safety programs and build upon food safety culture
- Novel ways to evaluate food safety other than the standard inspection.

Store employees, corporate, and regulatory personnel all have the same goal – to keep people safe and quality auditing programs are key to supporting that goal and fostering food safety culture.

RT19 Sweet and Saucy! The Role of Sugar and Other Important Considerations in the Classification and FDA Filing of Acidified Foods

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The classification of foods as per the requirements of the FDA's acidified food regulations (21CFR114/108.25) continues to cause confusion and disagreement among regulators, food and beverage producers, academia and food processing authorities. The reason for confusion is rooted on the passage in 21CFR114 stating that certain products that *…contain small amounts of low-acid food(s) and have a resultant finished equilibrium pH that does not significantly differ from that of the predominant acid or acid food"* are exempted from the requirements of the regulation. A critical point of contention is the designation by FDA of water and sugar as low-acid ingredients. Many in the scientific and food industry community disagree with this assessment and feel strongly that the buffering capacity of these ingredients should be an important component of the product classification process.

In a well-attended roundtable during the 2022 IAFP Annual Meeting, a representative of FDA provided insightful and important clarification as to the water debate. However, the debate around sugar as well as the overall classification of certain products persists, and its resolution is of tremendous importance to the industry. Many of these products such as syrups, dressings, and performance beverages are processed without a thermal process or are filled non-aseptically at ambient temperature. In these cases, a challenge study demonstrating that pertinent pathogens die quickly in the product must be submitted to the agency when filing the process. The design, execution and interpretation of these challenge studies are important considerations in this discussion.

The objective of this roundtable is to provide the audience with an important update on this subject, present the industry perspective, provide novel tools to measure buffering capacity, and provide guidance on the design, execution and interpretation of challenge studies aimed to facilitate the filing of non-thermally processed and/or non-aseptically ambient filled products.

RT20 Are We Meeting Our Targets? Healthy People 2030 and the National Effort to Drive Down Foodborne Illness in the United States

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JENNIFER MCENTIRE: *Food Safety Strategy, LLC, Frederick, MD, USA*
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The Healthy People (HP) initiative provides science-based national measurable objectives for improving the health of Americans. Updated in 10-year increments, it is led by the U.S. Department of Health and Human Services in collaboration with federal and non-federal stakeholders and subject matter experts. HP 2030 includes four core foodborne objectives focused on the incidence of *Salmonella*, Shiga-toxin producing *E. coli*, *Listeria*, and *Campylobacter* illnesses. There are also objectives related to antimicrobial resistance, consumer and retail food safety practices, and reducing outbreaks due to specific pathogen-commodity pairs.

This roundtable will facilitate lively discussion on the role of HP objectives and activities in national food safety efforts. In addition to providing some historical perspective, the roundtable will focus attention on current HP2030 activities. Panelists will describe how targets are set and measured, and where we are making progress and where we are lagging. The diverse panel will address a number of questions. How do these objectives and data inform national policy? How are they used by food producers, retailers, and consumer groups? What are the impacts of changing diagnostics, surveillance, and tools for prevention? What additional efforts are needed to meet HP2030 targets?

Healthy People has not been a recent subject of a conference session. The timing of a roundtable in 2024 is opportune because we are roughly halfway between the Healthy People 2020 and 2030 targets. This will be a good opportunity to discuss both the past and the future of Healthy People.

Learning Objectives:

- Describe the collaboration of CDC, FDA, USDA/FSIS, and Associations on the Healthy People Food Safety Topic Area.
- Obtain industry, academic, and consumer perspectives on how Healthy People is used in making decisions on reducing foodborne illnesses.
- Discuss forward-looking proposals, policies, initiatives, resources, and boots-on-the ground actions that are intended to drive down foodborne illness burden through Healthy People.

RT21 Strengthening the Frontline of Food Safety: Meeting the Growing Demand for Competent Auditors, Inspectors, and Assessors

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STAN OSUAGWU: *Home Chef, Chicago, IL, USA*
CLINT STEVENSON: *North Carolina State University, Raleigh, NC, USA*
ALLISON JENNINGS: *Albertsons Companies, Boise, ID, USA*
PHILLIP PIERCE: *NSF, Key West, FL, USA*
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Dire workforce shortages exist across food safety auditing, inspections, and assessment (AIA) occupations with vacancy rates between 10 and 15%. Contributing factors include attrition through retirement, competing job opportunities, and a general insufficient awareness of AIA career paths. Turnover rates for food safety inspectors are generally between 12–15% with certain agencies and geographic locations experiencing turnover rates as high as 20–25%. Inspector demand is expected to grow 9% over the next decade. Available candidate pools are so small that some regulatory agencies have waived the requirement of an undergraduate degree. The consequences of high vacancy rates and turnover in food safety AIA occupations are significant training costs passed onto consumers and taxpayers and inadequate oversight of the food supply chain.

The proposed roundtable will engage representatives from auditing, regulatory, foodservice, manufacturing, food retail, and higher education institutions in a discussion about the AIA workforce shortages and potential solutions. Topics will include root causes, impacts on the food industry, needs, and efforts and ideas to recruit and develop an AIA workforce prepared to protect our food supply. Session attendees of this roundtable discussion will gain an understanding of the magnitude of the AIA workforce shortage and opportunities to become engaged in the solution through various avenues to promote careers and enhance preparedness in food safety AIA.

RT22 Leveraging GS1 Standards and Advanced Data Carriers to Support FSMA 204 Traceability Requirements

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RENEE PERRY: *Culinary Collaborations LLC, Rochester, NY, USA*
MARGARET MALKOSKI: *National Fisheries Institute, McLean, VA, USA*

In November of 2022, The U.S. Food & Drug Administration (FDA) finalized their traceability rule for companies who manufacture, process, pack or holds certain high-risk foods under Section 204 of the Food Safety Modernization Act (FSMA).

Companies have one year and a half left to comply with this regulation and time is of the essence when it comes to implementing a traceability program. Food companies realize they cannot do this alone. Now more than ever, it is critical to understand what key industry players are doing to collaborate and align to meet common goals. GS1 Standards are playing a pivotal role in helping organizations on their compliance journey.

In this session, attendees will learn directly from those implementing traceability programs to meet FSMA 204 requirements and which specific tools they're using to achieve this.

Learning Objectives:

- Learn firsthand from traceability programs or pilots being implemented today
- Understand how companies are using Data Carriers such as barcodes for FSMA 204 Compliance
- Discover EPCIS 2.0 as a technology or as a language and structure everyone can use to capture and share traceability data required by the FDA
- Consider which Implementation Strategies would best fit their business needs
- Understand some Key Hurdles and pitfalls to avoid

Product traceability improvements are essential to protect and enhance the safety of our food supply. To that end, the U.S. Food & Drug Administration (FDA) recently finalized a new Traceability Rule, outlining next-level recordkeeping requirements for producers of high-risk foods under Section 204 of the Food Safety Modernization Act (FSMA).

This session will help attendees understand how they can collect, homogenize and share the necessary data, including mandatory Key Data Elements (KDEs) and Critical Tracking Events (CTEs) occurring within the supply chain, to meet the Final Rule requirements. Leading food producers will share results of their traceability pilots, providing insight into challenges, opportunities and implementation strategies. Attendees will learn how GS1 Standards and advanced data carriers can be applied to enable effective implementation and meet the January 20, 2026 FDA compliance deadline by establishing a common language for key product data to be captured, shared, retrieved, aggregated, and interpreted by all links in the supply chain.

RT23 Current and Novel Approaches to Food Source Attribution

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TINE HALD: *Technical University of Denmark, Lyngby, Denmark, Denmark*
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Foodborne illness source attribution enables us to assess which foods are most likely responsible for specific foodborne illnesses and outbreaks. Many sources of data are needed to make attribution estimates, including data from outbreak investigations, sporadic human illness data and contamination data from foods. Determining the sources of foodborne illness is important, to facilitate recalls and enable prevention strategies to be developed.

In 2007, the World Health Organization (WHO) established the Foodborne Disease Burden Epidemiology Reference Group, to provide estimates on the global burden of foodborne diseases and to strengthen the capacity of countries to improve food safety. A subcommittee on source attribution was established to assess which foods are responsible for specific foodborne illnesses. The subcommittee findings have been published in several reports.

While WHO provides attribution estimates globally, member states may also provide national or regional source attribution estimates. For example, in the U.S., the Interagency for Food Safety Analytics Collaboration (IFSAC), a partnership among the Centers for Disease Control and Prevention (CDC), the U.S. Food and Drug Administration (FDA), and the Food Safety and Inspection Service (FSIS) develops estimates of foodborne illness source attribution and produces and publishes annual Foodborne Illness Source Attribution estimates, focused on *Campylobacter, Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella*.

This roundtable will provide a forum for those working in source attribution to share their current approaches and discuss future possible approaches to improve source attribution and how to improve data sharing and collaboration among the different organizations who generate food safety data. These data may be used to inform strategic planning and decision-making, and evaluate the impact of interventions, such as new or revised regulations, policies, and performance standards.

RT24 The Required Evolution of Best Practices Based on Science for Fresh-Cut Produce

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CHANNAH ROCK: *University of Arizona, Maricopa, AZ, USA*
ANA ALLENDE: *CEBAS-CSIC, Murcia, Spain, Spain*
DREW MCDONALD: *Taylor Fresh Foods, Salinas, CA, USA*

An interactive discussion of the evolution and lack of evolution in best practices in the realm of Fresh Cut Produce. The panel will consider the premise that every illness or outbreak is a learning opportunity. This discussion will apply to the broader issue of food safety in general. The discussion will delve into the realities on the ground versus expectations and the balance between the quantity and quality of food safety efforts using interactive computer simulations to facilitate discussion. Simulations allow comparison of the relative mitigation power of various scenarios where actual execution would be impractical.

The Fresh Cut Industry is under constant pressure to do more pathogen testing to reduce consumer risk. Other practices have and could result in more food safety impact including ag water management, Good Agricultural Practice (GAP), the foundation sanitation programs of HACCP, and Processing. When new risks are identified, the preventative efforts need to evolve to meet the challenge. Our panelists will discuss how to use residual risk management to evaluate various options and not just add more.

Food safety budgets are limited, necessitating choices focused on eliminating risks and enhancing food safety. Discussion will include an examination of the defining qualities of best practices in ag water, GAP programs, the quality of harvest sanitation programs, the effectiveness of processing techniques, in-plant sanitation, and the strategic use of testing.

Furthermore, the discussion will explore the balance between testing raw materials and finished products and the role of a feedback loop that employs root cause analysis to eliminate recurring problems and improve best practices. Continuous improvement is achieved through cycles of observation, analysis, and learning. While the industry regrets each illness, it must reassert its efforts to follow the paths of Deming, Juan and Crosby to proactively prevent problems. Testing alone, despite it emotional appeal, cannot guarantee food safety. To improve is to change; to be perfect is to change often.

Technical Abstracts

T1-01 Strain-Level Persistence and Multi-Species Biofilm Formation of Microbes in a Meat Processing Facility

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Introduction: Biofilms in meat processing facilities contribute to meat spoilage and impede the sanitization efficacy, potentially leading to the permanent persistence of spoilage microbes with facilities.

Purpose: To characterize the microbial community in a meat processing facility at strain-level identification and determine the overlap between isolates from different sites in the facilities and meat products at two sampling times, six months apart. Isolates were used to reconstitute multi-species biofilms to assess their biofilm formation and composition.

Methods: Bacterial isolates were collected from 116 sampling sites over two visits at a 6-month interval, including non-food contact surfaces, food contact surfaces, meat at the time of processing and at the end of the shelf life. Each isolate was classified through genome sequencing to determine their species-level taxonomy. Strain-level persistence of meat spoilage isolates was revealed through phylogenetic analysis based on the core genome phylogenetic tree. Reconstitution of multi-species biofilm from 10 sampling sites was tested on stainless steel coupons at 25°C and 4°C for 6 days (n=3). Biofilm formation ability and the composition of each biofilm community were analyzed through crystal violet staining, conventional plating and full-length 16S rRNA gene sequencing.

Results: Characterization of 2116 isolates from 55 different genera revealed a large diversity of microbes within the facility. Whole genome sequencing demonstrated that microbes on meat originate from the processing facility and persist despite sanitization efforts. Strains of *Carnobacterium maltaromaticum* persisted over a period of 6 month across sampling sites and time, particularly stemming from floor drains in the cooler room. Other spoilage microbes originated from food-contact and non-food contact environments of packaging area in the fabrication room. Reconstituted microbial communities formed denser biofilms at 4°C than 25°C.

Significance: Microbial populations permanently reside within meat processing facilities as a source of transmission of spoilage and pathogenic microbes. This finding underscores the need for improved and site-specific sanitization strategies.

T1-02 Salmonella Surveillance in Broiler Breeder Flocks with Rodent Control

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❖ ***Developing Scientist Entrant***

Introduction: Successful *Salmonella* control during broiler production relies on robust biosecurity and an appropriate surveillance platform, which includes monitoring breeder flocks.

Purpose: This study was designed to determine the incidence of multi-serovar *Salmonella* populations in broiler breeder flocks, and to highlight the importance of maintaining rodent control to limit *Salmonella* introduction.

Methods: Across two broiler complexes (A, B), 15 pullet (five farms) and 13 breeder houses (seven farms) were sampled over a 65-week production period. Pullets were sampled at weeks 14 and 21, then breeders sampled every four weeks. Two bootsock pairs were collected from each house and cultured for *Salmonella* (n=394). Rodents (mice plus roof and Norway rats; n=355 carcasses across 49 composite samples) were captured from farms and tested for *Salmonella*, along with bait station swabs (n=33).

Results: Overall *Salmonella* prevalence in pullets was 17% (11/64), although only houses in Complex B were positive (6/7 houses). All 13 breeder houses were *Salmonella*-positive at least once; the overall prevalence was 34% (62/182) and 54% (80/148) in Complexes A and B, respectively. A generalized additive mixed effects model showed the expected marginal mean *Salmonella* prevalence peaked ~38 weeks. Deep serotyping revealed 38% (53/140) of bootsocks contained multiple serovars. The number of serovars differed between Complex A (five serovars) and B (15 serovars)(Shannon diversity index plus Hutcheson t-test ($p < 0.05$)). In rodents, 35% (17/49) of composite samples and 9% (3/33) of bait station swabs were positive, and six serovars were identified. Four rodent serovars were also found in bootsock samples from the same farms/houses, with two serovars matching *Salmonella* subtypes between bootsocks and rodents.

Significance: This longitudinal characterization of *Salmonella* highlights differences across broiler complexes, which may be attributed to management practices as Complex A employs a 3rd party integrated pest control company while Complex B relies on growers.

T1-03 Evaluation of *Staphylococcus aureus* Growth in Slow-Cooked Turkey Meat Products

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Introduction: USDA FSIS Appendix-A recommends that meat dwell ≤6 hours from 50 to 130°F during cooking to limit a conducive environment for *Staphylococcus aureus* to grow and produce heat-stable enterotoxins. *S. aureus* growth of ≥3-log is a public health concern.

Purpose: To assess *Staphylococcus aureus* growth in turkey meat formulations during heating from 50 to 130°F in 9.3-hours, representing a HACCP deviation case in a commercial establishment.

Methods: Irradiated ground turkey meat formulated with either liquid vinegar @ 2% (V), lactate-diacetate @ 2.5% (L), or without antimicrobial (C) were evaluated. An individual experimental unit consisting of a 5-g-portion of meat in a cookable plastic bag was inoculated with a four-strain cocktail of *S. aureus*, then spread thin in the bag. Fifty units per formulation were heated together in a water bath to a target time-temperature profile. *S. aureus* counts were enumerated on Baird-Parker agar from five inoculated units at time 0, and three units each at 4.5, 6.0, 7.0, 8.0, and 9.3 hours. Three replicate trials were performed. Counts were transformed to log scale and reported as mean±SD of the trials.

Results: The formulations had pH, moisture, and salt content of 6.74±0.08, 76.4±0.6%, and 0.6±0.1%, respectively. Formulations reached temperatures of 50, 65, 79, 89, 98, 106, 113, 120, 124, and 128°F from 0 to 9 hours, and attained 130°F at 9.3 hours. *S. aureus* count at time-0 was 2.8±0.3-log CFU/g. Maximum growth of 0.5±0.3, 1.3±0.3, and 1.6±0.2 log was observed in the 6th hour in formulations V, L, and C, respectively ($p<0.05$). Thereafter, the counts decline ($p<0.05$). When compared with available validated models, UW Therm and DMRI Staphtox Predictor, after adjusting the temperature limitations per Appendix-A guideline, 4.3- and 3.6-log increase, respectively, was estimated.

Significance: UW Therm and DMRI Staphtox Predictor provided safe predictions; however, temperature and formulation limitations in these models may result in overly conservative predictions.

T1-04 Comparative Genomic Analysis of a Bacteriophage Preparation Targeting *Listeria monocytogenes* and Its Efficacy on Italian-Style Ham

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Introduction: *Listeria monocytogenes* is a significant food safety concern and is considered an adulterant in ready-to-eat meat. Bacteriophages have recently gained significant interest as a natural, safe, green, and effective method to reduce foodborne pathogen contamination of various foods.

Purpose: Comparative genomic analysis of the component bacteriophages of a commercial preparation "ListShield" was performed. Secondly, efficacy of bacteriophage treatment to reduce *L. monocytogenes* on contaminated Italian-style ham was evaluated.

Methods: Bacteriophages were sequenced on a MinIon sequencer (coverage depth >2400X), assembled using Flye assembler and polished using Pilon and Medaka. Assembled linear genomes were compared with Listeria phage A511 using EasyFig. Additionally, whole genome assemblies of a total of 100 Listeria phage genomes available in NCBI database were clustered using VICTOR by comparing amino acid sequences. Efficacy of bacteriophage application was evaluated in two independent tests by inoculating 12-14 Italian-style ham pieces per test with either a single strain of serotype 4b or a mixture of serotypes 1/2a and 4b on Italian style ham. Bacteriophages were applied at an application rate of 2 mL/lbs yielding ≈$4.5x10^6$ PFU/g.

Results: Comparative genome analysis showed that the Listeria phage LMTA-148 was the most diverse phage in the bacteriophage preparation. VICTOR analysis identified 5 previously reported Orthoclusters. Bacteriophages in commercial preparation were grouped into two subclusters within the Orthocluster I, indicating these phages are distinct from other identified Listeria phages. The bacteriophages were highly effective in reducing *L. monocytogenes* contamination on ham, reducing ≈1.0 log in 15 min and up to 2.0 log after 24h treatment. The preparation did not show a pronounced preventative effect when bacteriophages were applied before *L. monocytogenes* contamination.

Significance: The comparative genome analysis suggests that the six phages in commercial preparation are distinct from other Listeria phages. The efficacy data demonstrated that phage biocontrol is an effective intervention for managing *L. monocytogenes* contamination of Italian-style ham and possibly other ham products.

T1-05 Effect of Nisin on the Growth of *Bacillus cereus* From Spores in Pasteurized Liquid Whole Eggs

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Introduction: Nisin is a bacteriocin produced by food-grade lactic acid bacteria (LAB) *Lactococcus lactis* subsp. *L. lactis is* often efficient against gram positive microorganisms. Using nisin in liquid whole eggs (LWE) enhances the safety of eggs against *Bacillus cereus* and increases their shelf-life.

Purpose: Investigate the inhibitory effects of nisin on the outgrowth of *B. cereus* spores in LWE and develop a predictive model under varying temperatures and concentrations of nisin.

Methods*:* Pasteurized LWE egg was formulated to contain different concentrations (0, 2, 4, & 6.25 ppm) of nisin. The eggs were inoculated with a four-strain cocktail of *B. cereus* spores (pre-heat treated at 85 °C; 30 min) to obtain initial inoculum concentration of 2.3-2.8 log CFU/ml. The growth/inactivation data of *B. cereus* under varying temperatures (15, 25, 35, 40, & 45 °C) was collected. The Baranyi model was used as a primary model to fit the data, and the polynomial model was fitted as a secondary model. Model fit parameters, including Root Square Mean Error (RMSE) and R^2 were used to evaluate the primary and secondary model fitting.

Results: The inhibitory effect was significantly (p<0.05) higher in LWE containing 6.25 ppm nisin with lag phase duration (λ) extending to 25,7.4, 4, 5.8 & 3.6 days at 15, 25, 35, 40 & 45 °C, respectively as compared to control samples with λ 26.7, 4.6, 2.2, 2.1 & 4.6 hours at respective temperatures. The maximum growth rate (u_{max}) followed the trend of modified Ratkowsky model, and the maximum value was calculated at 40°C. However, the effect in u_{max} was not significant with different concentrations of nisin.

Significance: Use of nisin facilitates the inhibition of *B. cereus* and increases the shelf-life of LWE eggs at abuse and optimum temperatures. Additionally, the model developed can be used to evaluate the effects of nisin on growth of *B. cereus* in LWE during storage and distribution.

T1-06 Exploring Genomic Differences between Strong Biofilm-Forming *Salmonella* Enteritidis Strains and Strains in the Public Database

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Introduction: *Salmonella* Enteritidis has been a persistent cause of human illnesses worldwide. Biofilms can promote the growth and survival of *Salmonella*.

Purpose*:* To conduct a genomic comparison between 15 *S.* Enteritidis strains with known biofilm formation abilities and 76 *S.* Enteritidis from public genomes recovered from poultry

Methods*:* 15 S*S.* Enteritidis E strains (14 from chicken products, 1 from bovine) with intermediate to strong biofilm-forming ability, positive for curli and cellulose production at 20°C, were contrasted with 76 *S.* Enteritidis genomes sourced from various origins: chicken products (n=10), chicken organs (n=16), chicken carcasses (n=1), egg house (n=2), chicken feces (n=16), and non-specified avian origin (n=31), collected from 1986 to 2022. A *S.* Enteritidis reference strain was included. The comparative systems genus-specific families (PLfams) analysis was conducted using the BV-BRC pipeline.

Results*:* Within 223 genes screened, 116 genes associated with biofilm formation, fimbriae, flagella, curli and cellulose-related proteins were found in all 15 SE strains. Among 131 missing genes, some are reported to be important for biofilm formation, such as AdrA (positively regulates cellulose synthesis). Compared with genomes from public databases, 88.8% (103/116) of the genes were all present. The absent genes (11.2%) corresponded to FimZ; BscE; SafB, FlgA, YehA, YehC, PilQ, AdrA, BpaA, and YcgR. Regarding virulence genes, 89 genes linked to type I to type VI secretions systems were present in all 15 SE strains. Among the public genomes, 50 strains carried 89 out of 91 genes were all present, except for Type IV secretion system genes VirB4, VirB2 and Type VI VasI, which were exclusive to the public genome and absent in the 15 strains.

Significance: Gene profiles associated with strong and intermediate biofilm formation in *S.* Enteritidis strains were commonly found in publicly available *S.* Enteritidis genomes. This suggests biofilm formation may be a prevalent trait among *S.* Enteritidis strains and could play a role in bacterial persistence.

T1-07 Metabolomics Profiling to Investigate the Resuscitation of Viable-But-Nonculturable *Campylobacter jejuni* in Embryonated Chicken Eggs

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Introduction: *Campylobacter jejuni* is a major foodborne pathogen and it can enter a viable-but-nonculturable (VBNC) state to survive under stresses. VBNC *C. jejuni* is able to resuscitate in embryonated chicken eggs, posing a serious health risk. However, the mechanisms underlying the resuscitation of VBNC *C. jejuni* in embryonated eggs has not been well understood.

Purpose*:* This study aimed to explore the potential compounds in embryonated chicken eggs that contribute to the resuscitation of VBNC *C. jejuni* using untargeted metabolomics.

Methods*: C. jejuni* strains ATCC33560 and F38011were induced into VBNC state at 4°C. VBNC cells were aseptically inoculated into the chicken eggs at different embryogenic days (D) of D1, D3, D5, D7, D9 and D11, respectively, to test the resuscitation rates. As different egg groups demonstrated different capabilities of promoting VBNC resuscitation, untargeted metabolomics was further performed using UHPLC-Q/TOF at both positive and negative modes to evaluate the compositional variations between eggs at early (i.e., D1 and D3) and later developmental stages (i.e., D9 and D11).

Results*:* Resuscitation rate of both *C. jejuni* strains gradually increased from 0 to 90% when tested in eggs at embryogenic stages from D1 to D11. Eggs at early stages (D1 and D3) were noticeably separated from late-stage eggs (D9 and D11) using principal component analysis. A total of 87 metabolites

were significantly (p< 0.05, fold change >2) increased in the D9-eggs compared to D3-eggs, and some may contribute to the VBNC resuscitation, such as fumaric acid, L-glutamine and 2-oxobutanoate. Pathway analysis indicated that nitrogen metabolism and amino acids metabolism (*e.g.,* glutamate and serine) in VBNC *C. jejuni* might be enhanced during the resuscitation in eggs.

Significance: The knowledge from this study can facilitate a better understanding of the resuscitation mechanism of VBNC *C. jejuni* and provide insights to the development of novel recipes for recovering and testing VBNC pathogens.

T1-08 Prevalence and Antimicrobial Resistance of *Salmonella* and *Campylobacter* Isolated from Retail Chickens in Saudi Arabia

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Introduction: *Salmonella* and *Campylobacter* are the most common foodborne bacterial pathogens isolated from poultry meat worldwide. In Saudi Arabia, over a third of *Salmonella* outbreaks between 2014 and 2018 were linked to poultry meat consumption.

Purpose: This study aimed to determine the prevalence and antimicrobial resistance of *Salmonella* and *Campylobacter* in broiler meat in Saudi Arabia.

Methods: A total of 106 chicken samples from nine brands, including three large farms, three medium farms, and three small farms, were purchased from major food stores between October 2022 and February 2023. The neck skin of each broiler chicken sample was removed, placed in a sterile plastic bag, and then the chicken carcass was rinsed with buffered peptone water. The homogenized samples were then incubated in specific broths: Rappaport Vassiliadis soya broth for *Salmonella* at 42°C for 24-h, and Modified Preston media with supplements for *Campylobacter* at 37°C for 48-h under microaerophilic conditions. Following incubation, suspensions were streaked onto media agars selective for each pathogen. Serological tests for Salmonella were carried out following the characterization and biochemical analysis of colonies, whereas *Campylobacter* isolates were subjected to oxidase and motility mode testing. Molecular characterization for both pathogens was conducted using Conventional PCR. Isolates were also assessed for antimicrobial susceptibility to 11 clinically important antibiotics.

Results: *Campylobacter jejuni* was isolated from 26.42% and C. coli from 9.43% of samples. *Salmonella* was present in 9.43% of samples, and all *Salmonella* isolates belonged to *S.* Enteritidis. Multi-drug resistance was observed in 100% of *C. jejuni* isolates, 90% of C. coli, and 70% of *S.* Enteritidis. The majority of positive samples were found in chickens produced by small farms, indicating a need for enhanced control measures against these foodborne pathogens in small farms.

Significance: This study is the first to evaluate the prevalence of *Salmonella* and *Campylobacter* in retail chicken in Saudi Arabia.

T1-09 The Detection and Molecular Characterization of *mcr-1*-Positive *Escherichia coli* Isolated from Poultry Meat

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Introduction: Previously, we highlighted the colonization of pre-harvest broiler chickens with multi-drug-resistant *Escherichia coli*, which also harbored the mobile genetic determinants (*mcr*) that encode resistance to colistin, a last-resort antibiotic.

Purpose: Despite the notable prevalence of *mcr* in pre-harvest poultry, data on *mcr* in postharvest chicken meat are very limited in Lebanon. Therefore, this study investigates the dissemination of *mcr* in retail chicken meat in Lebanon.

Methods: Skinless chicken breast samples (n=151) were aseptically collected from different retail markets (n=25) and butcheries (n=26) in Lebanon. The microbiological load of *Escherichia coli* was assessed by homogenizing the chicken samples (~25 g) in buffered peptone water (225 mL), followed by serial dilution (10-folds), and spreading (100 µL) of the homogenate on RAPID'E. coli 2 Agar. Distinctive *E. coli* isolates were selected to analyze their antibiotic resistance profiles (ABR) using the disk-diffusion assay. A subset of isolates from retail markets (n=16) and butcheries (n=26) exhibiting unique ABR profiles underwent a comprehensive characterization using Whole Genome Sequencing (WGS) to evaluate their resistome. To investigate the persistence of these isolates, biofilm formation was assessed using the crystal violet assay.

Results: Five *mcr-1*-positive *E. coli* were identified using WGS and confirmed by gene-specific PCR analysis. These isolates were retrieved from samples collected from four different butcheries and exhibited a high microbiological load of *E. coli* (ranging from 3.5 x 10^2 to 7.15 x 10^3 CFU/g). In addition to *mcr-1*, the isolates harbored up to 10 acquired ABR genes that encoded resistance to different antibiotics, including cephalosporins, sulfonamides, aminoglycosides, and tetracyclines. Furthermore, the isolates exhibited a medium to strong biofilm-forming capacity (OD_{600} 0.6 -1.2). Taken together, our data highlighted the potential for ABR *E. coli* to persist in the poultry production chain.

Significance: The detection of *mcr-1* in *E. coli* on chicken meat emphasizes the need for effective monitoring and intervention strategies against the misuse of critical antibiotics in agriculture in Lebanon.

T1-10 Ultra-Fine Ozone Bubbles: An Effective Chill Tank Treatment to Reduce *Salmonella* Enteritidis Cross-Contamination in Poultry Carcasses without Affecting Product Color

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Introduction: The chill tank act as a source of *Salmonella* Enteritidis cross-contamination between poultry carcasses. Therefore, there is a need for developing effective intervention strategies for reducing *S.* Enteritidis cross-contamination in chill tank.

Purpose: This study investigated the efficacy of water containing Ultra-fine ozone bubbles (UFOB) in reducing *Salmonella* Enteritidis on poultry carcass. In addition, effect of UFOB treatment on carcass color was studied.

Methods: UFOB were produced in water maintained at 4°C using an ozone injected-nanobubble generator. The characteristics of UFOB and dissolved ozone levels were measured using NanoSight300 and ozone detection kit, respectively. Chicken skin (4 x 4 cm) was spot-inoculated with a 4-strain cocktail of *S.* Enteritidis (7 log CFU/sample) and allowed to attach for 120 min. One *S.* Enteritidis inoculated skin along with 9 non-inoculated skins were dipped in refrigerated DI water or UFOB water for 30 min. After treatment, surviving *S.* Enteritidis on chicken skin was enumerated on XLD agar. All experiments had duplicate samples, repeated thrice, and analyzed using student t-test (p<0.05). The color measurement was carried out using a chroma meter.

Results: The dissolved ozone concentration in UFOB water was ~9 ppm. Bubble concentration was ~10^8/ml with size ~100-200 nm. Dipping of non-inoculated samples with inoculated sample in DI water resulted in 100% cross-contamination (10 samples positive out of 10). Dipping in UFOB water significantly reduced cross-contamination by 50% (5 samples positive out of 10). *S.* Enteritidis load on skin treated with UFOB water reduced by ~1 log CFU/sample as compared to control (p<0.05). The UFOB water treatment did not affect the color of chicken skin. The effect of UFOB water dip on meat quality and consumer acceptability is currently underway.

Significance: UFOB water could be used for controlling cross contamination of *S.* Enteritidis in carcass chilling tank without affecting the color parameters.

T1-11 Food Spoilage Troubleshooting: From PCR Development to Process Adaptation, A Success Story of Spoilage Yeast Management in Food Production Facility

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Introduction: A French food manufacturer was concerned with recurrent organoleptic alteration of raw vegetables tartares. Whereas low pH of the finished product was expected to prevent microbial growth, yeasts were regularly isolated from defective swollen products. Provied natural background yeasts, classical ISO 21527-1:2008 enumeration was not sufficient to identify and control the contamination sources.
Purpose: The present study reports a successful step-wise troubleshooting including (i) development and validation of a specific isothermal PCR (LAMP), (ii) screening of production environment and raw materials and (iii) process adaptations.
Methods: ISO 21527-1:2008 enumerations were performed on swollen defective products and the isolated colonies were identified by 16S ribosomal sub-unit (bacteria) or ITS sequencing. Based on literature review, the putative spoiler was identified as *K. barnettii*. A specific isothermal PCR-based (LAMP) method was developed to investigate the presence of *K. barnettii* in various raw materials and surfaces in the production facility and to implement relevant corrective actions.
Results: Despite the presence of other contaminants, the main yeast isolated from defective product was *Kazachstania barnettii*, which is known to produce spectacular swollen packaging effect. Specific detection method was successfully developed with a level of detection (50%) below 10 CFU/sample regardless of the tested matrices. Among the tested raw materials and surfaces, only onions, shallots and surfaces sampled from bowl cutter (used to process the latter vegetables) were significantly contaminated by *K. barnettii*. Implementation of a new blanching step for contaminated raw vegetables, reinforced cleaning and disinfection procedure for the bowl cutter and regular monitoring of *K. barnettii* in the facility drastically reduced the occurrence of the defect.
Significance: Managing food spoilage resulting from yeast contamination can be extremely challenging for food business operators due to multiple contamination sources, preservative resistance, and the lack of specific detection methods. The described stepwise and custom PCR approach was helpful to successfully identify and control the contamination sources in the facility.

T1-12 Unlocking the Role of the Novel RacRS Regulatory System in the Pathogenesis of *Campylobacter jejuni*

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Introduction: Human infection with *Campylobacter jejuni* can result in one of two different diarrheal manifestations: a bloody, inflammatory diarrhea or a watery diarrhea. However, little is known about the underlying genetics, pathogenesis, or host factors involved in these diarrheal manifestation differences.
Purpose: Previous research in the Cooper laboratory has identified unique non-synonymous SNPs on either the *rac*R or *rac*S genes only in strains associated with bloody/inflammatory diarrhea. These genes are part of the two-component signaling pathway called RacRS that is necessary for colonization of the chicken's intestinal tract. To date, there has been no research investigating the full role that RacRS has on *C. jejuni* pathogenesis in general or different diarrheal manifestations. We hypothesis that RacRS influences the type of diarrheal manifestation generated by a particular *C. jejuni* strain.
Methods: To investigate this, *C. jejuni* double knockout mutants were made in two strains associated with watery diarrhea (S3 and D42) and in two strains associated with bloody/inflammatory diarrhea (M129 and 81-176) using electroporation. All RacRS mutants, complementation strains and wildtype *C. jejuni* strains were then screened for several phenotypic and/or virulence assays including biofilm production, motility, generation time, invasion, attachment, and cytolethal distending toxin production.
Results: These studies found statistically significant differences between the wildtype and the RacRS mutant in motility (M129 p value = 0.04735, D42 p value = 1.847e-05) and attachment (M129 p value = 0.02452, D42 p value = 0.03187) As well as invasion in INT-407 cells (M129 p value = 0.002179, D42 p value = 0.006273) and in CaCo-2 BBE cells (M129 p value = 0.002292).
Significance: These results suggest that RacRS has a role in virulence, particularly attachment and invasion, which we have previously demonstrated is only important for strains associated with bloody, inflammatory diarrhea manifestations. This research is a critical first step in unlocking the role RacRS has in *C.jejuni* pathogenesis and particularly the diarrheal outcome in patients.

T2-01 Assessing *Enterococcus faecium* NRRL B-2354 as a Surrogate for *Listeria monocytogenes* during Sanitizer Interventions in Simulated Apple Dump Tank Water

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Introduction: Sanitizers are widely incorporated in commercial apple dump tank systems to mitigate cross-contamination, to validate the efficacy of these interventions in the presence of organic matter, a reliable surrogate is required to predict the behavior and susceptibility of *Listeria monocytogenes*.
Purpose: To verify the suitability of *Enterococcus faecium* NRRL B-2354 as a surrogate for *L. monocytogenes* during sanitizer interventions in dump tank water systems.
Methods: Granny Smith apples were inoculated with respective bacteria at 6.0 log, then subjected, either individual or alongside uninoculated apples, to sanitizer solutions in simulated dump tank water (SDTW) at 1000 ppm chemical oxygen demand (COD) for 2- and 5-min. Additionally, *L. monocytogenes* and *E. faecium* inoculum at 6.0 log were challenged in SDTW treated with sanitizers, with or without the addition of uninoculated apples, for up to 5-min.
Results: *E. faecium* on apples exhibited statistically ($p \leq 0.05$) equivalent susceptibility to *L. monocytogenes* when exposed to chlorine (25-100ppm) and PAA (20-80ppm) treatments in SDTW with 1000 ppm COD, resulting in 0.2-1.1 and 1.1-1.7 log reductions, respectively. Chlorine and PAA interventions demonstrated statistically similar efficacy against both bacteria in SDTW, yielding 0.7-4.8 and 2.0-5.0 log reduction in water with 1000 ppm COD, respectively, and 0.3-4.0 and 1.9-5.0 log reduction in water with 4000 ppm COD. No statistically significant difference was observed between the transference of both bacteria from inoculated apples to uninoculated apples and the water.
Significance: The data suggest *E. faecium* is a viable surrogate for *L. monocytogenes* in dump tank washing system, which could be used to predict the anti-*Listeria* efficacy of chlorine and PAA interventions during commercial apple processing.

T2-02 Comparison of Two Disinfectant Spraying Application Methods to Assess *S. aureus* Cross-Contamination Risk on Food Contact Surfaces

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Introduction: Different spraying application methods have been used to clean and disinfect food contact surfaces; limited data is available on application methods and efficacy.
Purpose: The purpose of this study was to evaluate hygiene outcomes of different spraying methods used with diverse products and wipe substrates and their ability to remove *S. aureus* from contaminated surfaces.

Methods: Spray surface and wipe and spray cloth and wipe methods were compared on a two m^2 Formica board. The board was marked with 100 cm^2 inoculation and sampling zones every 0.5 m. Non-woven, cotton, and microfiber cloths were tested with two disinfectants, two sanitizers, and a neutral cleaning product. The inoculation zone was inoculated with $5x10^8$ CFU *S. aureus* and dried for one h. Each material and product combination were tested. After five min contact time, sample zones were swabbed and plated on tryptic soy agar for 24 h at 37°C. Testing was done in triplicate; statistical analysis was completed in SAS.

Results: There were limited differences between spraying application methods. Application method significantly impacted recovery at the inoculation zone (p<0.0001), but not across the surface (p=0.8952). Product efficacy and wiping substrate significantly differed at the inoculation zone (p<0.0001). The hydrogen peroxide-based product (4.39 log CFU) and microfiber (3.66 log CFU) resulted in greatest log reduction. The neutral cleaner resulted in high cross-contamination irrespective of wiping material. Wiping materials had the highest bacterial load (5.22 log CFU); the sampling zone furthest from the inoculation zone had the least (1.87 log CFU).

Significance: Both methods with antimicrobial products effectively reduce S. aureus and limited cross-contamination on surfaces. Disposal of single use and handling reusable wiping materials should be considered to prevent cross-contamination.

T2-03 Effectiveness of Chlorine and Peroxyacetic Acid in Controlling *Listeria* Cross-Contamination during Pilot-Scale Dump Tank Apple Processing

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❖ ***Developing Scientist Entrant***

Introduction: Chlorine and peroxyacetic acid (PAA) utilized in commercial apple dump tanks is crucial in preventing microbial cross-contamination, however, their anti-*Listeria* efficacy assessed through bench-top experiments may not accurately represent the practical efficacy due to significant variations in processing parameters.

Purpose: To validate the efficacy of chlorine and PAA against *Listeria* on apples and assess potential cross-contamination during pilot dump tank processing with simulated dump tank water (SDTW).

Methods: In the apple-to-apple cross-contamination scenario, groups of 8 inoculated apples (~6.0 log CFU/apple *Listeria innocua*) and 32 uninoculated apples were treated with chlorine (25-100 ppm) and PAA (20-80 ppm) for up to 10 min in a pilot dump tank with 102 L of SDTW. In the water-to-apple cross-contamination scenario, 40 uninoculated apples were introduced to SDTW with 6.0 log *L. innocua* treated with chlorine or PAA at specified concentrations.

Results: Chlorine and PAA exhibited concentration- and contact time-dependent anti-*Listeria* efficacy. Chlorine (25-100 ppm) treatment for 0.5-10 min in SDTW with 1000 ppm chemical oxygen demand (COD) resulted in a reduction of *L. innocua* by 0.4-1.1 log on inoculated apples and 3.5-5.4 log in wash water, with 0.4-3.5 log of *L. innocua* transferred to uninoculated apples. PAA at 20-80 ppm led to a reduction of 0.5-1.5 log on inoculated apples, 0.8-4.3 log in SDTW, and a transference of 3.5-4.2 log to uninoculated apples. Increasing the organic load in SDTW from 1000 to 4000 ppm COD reduced the effectiveness of chlorine and PAA in mitigating *Listeria* cross-contamination.

Significance: This study provides valuable insights on the efficacy of sanitizer treatments during dump tank processing of apples.

T2-04 Evaluation of Cloth-in-Bucket and Pre-Wet Cleaning and Sanitizing Methods on *S. aureus* Cross-Contamination on Food Contact Surfaces

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Introduction: Various cleaning and disinfecting methods are used to mitigate pathogens on food contact surfaces; limited data are available on most effective methods to prevent cross-contamination.

Purpose: The purpose of this study was to investigate hygiene outcomes of cloth-in-bucket and prewet methods with a range of chemistries and wipe substrates and their ability to minimize cross-contamination.

Methods: Cloth-in-bucket and prewet cloth methods were compared using a two m^2 Formica board. The surface was marked with 100 cm^2 inoculation and sampling zones every 0.5 m for two m. Non-woven, cotton, and microfiber cloth were tested with chlorine-, quat-, and hydrogen peroxide-based disinfectant and sanitizers, and a neutral cleaner. The inoculation zone was inoculated with $5x10^8$ CFU *S. aureus* and dried for one h. The surface was wiped with each material-product combination. After a 5 min contact time, sample zones were swabbed using sponges with neutralizing buffer. Samples were plated on tryptic soy agar and incubated for 24 h at 37°C. Testing was done in triplicate; statistical analysis was completed in SAS.

Results: Overall, cloth-in-bucket and prewet cloth methods performed similarly. Differences in product performance varied at the inoculation zone and subsequent sampling zones; wipe substrate was significant (p<0.0001). At the inoculation zone, the hydrogen peroxide-based sanitizer reduced more bacteria (6.83 log CFU). The microfiber cloth resulted in the most removal of bacteria (3.34 log CFU). The cotton cloth resulted in the most cross-contamination (2.77 log CFU) compared to the microfiber and non-woven cloth. Wiping materials had the most contamination on average (4.50 log CFU; (p<0.0001), especially when compared to two m from the inoculation zone (1.75 log CFU).

Significance: Both the cloth-in-bucket and prewet methods effectively reduced the amount of S. aureus at the inoculation zone when used with disinfectant products regardless of wiping materials. Material and chemistry combined limited cross-contamination.

T2-05 Factors Affecting the Adhesion of Flour Particles to Stainless Steel Surfaces and Vacuum Dry-Cleaning

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Introduction: Recent outbreaks linked to flour-based products have highlighted the need for improved cleaning methods in low-moisture environments. Factors affecting flour particle adhesion must be better understood.

Purpose: The objectives of this study were to quantify: (1) triboelectric charge build-up in flour under different relative humidities (20, 40, 60%), (2) impact of powder size (US standard No. 60 – 80 or 80 – 100 mesh), electrostatic charge (charged and uncharged), and relative humidity on powder adhesion to electropolished 304 stainless steel coupons (8 × 8 × 0.2 cm), and (3) impact of vacuum nozzle angle (0, 45, 90° relative to the surface) on cleaning effectiveness.

Methods: Chargeability (nC) of flour samples flowed through a stainless-steel pipe was assessed using Faraday cup electrometry. The adhesive force of the flour particles was measured using a custom-built impact tester. The surface cleanliness after vacuum treatments was assessed using ATP (adenosine triphosphate) swabs and a luminometer.

Results: Charged flour samples at 20% RH exhibited significantly higher charge compared to those at 40 and 60% RH (p<0.001). Within the 60 – 80 mesh range, charged flour had higher adhesion percentages than uncharged samples at both 20 and 40% RH (p<0.001). However, in the 80 – 100 mesh range, charged flour did not show a significant difference in adhesion when compared to uncharged samples (α=0.05). Additionally, at 60% RH, surface residues measured by ATP were significantly lower for 90° nozzle angles than for 0° across both 60 – 80 mesh (p=0.031) and 80 – 100 mesh (p=0.043) flour size ranges.

Significance: Dry conditions (20-40% RH) produce more triboelectric charge which significantly affect larger-sized (<80 mesh) wheat flour particle adhe-

sion to stainless steel surfaces. In a humid environment (60% RH), it is recommended to keep the vacuum nozzle perpendicular to the surface for optimal cleaning effectiveness.

T2-06 Formation and Control of *Listeria monocytogenes* Biofilms on Various Food Processing Surfaces

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Introduction: Biofilms are complex microbial structures, embedded in an extracellular polymeric substance. The ability of *Listeria* to form biofilms is directly related to its persistence and potential contamination in food premises. Thus, it is crucial to understand *Listeria* biofilm formation and control.

Purpose: Assess the growth of *L. monocytogenes* biofilms on different surfaces representative of food processing facilities and evaluate the efficacy of commercially available sanitizers and UV-C light.

Methods: A three-strain cocktail of *L. monocytogenes* was grown for 96 hours at 25 ±2°C in a CDC Biofilm Reactor to form mature biofilms on stainless steel, nylon, high density polyethylene (HDPE), Polyvinylchloride (PVC), and Teflon. Biofilms on coupons were treated with peracetic acid (120 ppm), Silver Dihydrogen Citrate (4%), lactic acid (4%), and UV-C light (254 nm) alone or simultaneously for 1- or 5-min. Coupons were placed in D/E neutralizing broth, and attached cells dislodged by vortexing and sonication. Cells were enumerated by serial dilution and spread plated. Confocal images were obtained before and after treatments. Experiments were conducted in triplicates per sanitizer and surface. Results were considered significant at $P<0.05$.

Results: Stainless steel, Nylon, PVC, HDPE, and Teflon coupons resulted in initial *L. monocytogenes* populations of 6.70, 7.04, 8.65, 7.17, and 8.75 log CFU/coupon, respectively. All treatments significantly reduced *L. monocytogenes* biofilms independently by surface type. There was no difference between the 1 min and 5 min treatment time when UV-C was applied alone ($p>0.05$). Overall, the lactic acid and UV-C simultaneous application for 5 min was found to be the most effective across surfaces ($p<0.05$). Images displayed surface cleanability and changes in architectural structure of microbial communities' aggregates before and after sanitizer treatments.

Significance: This study provides insightful information on the use of a combined sanitizing approach on various food-processing surfaces based on cleanability, topographical and architectural characteristics.

T2-07 Biofilm Matrix of *Salmonella* Enteritidis Phage Type 30 Grown Using the CDC Biofilm Reactor: Desiccation, Rehydration, and Sensitivity to Antimicrobial Oils

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Introduction: *Salmonella* spp. are environmentally persistent pathogens, among which biofilm-forming strains are of particular concern. Uncontrolled use of water is undesirable across low-water activity (aw) food processing environments, calling the need for advancements in dry cleaning and sanitation.

Purpose: To assess the efficacy of oil-based antimicrobials against hydrated and desiccated *Salmonella* biofilms.

Methods: *Salmonella enterica* subsp. *enterica* serovars were grown on LB agar without salt supplemented with Congo Red, Coomassie Brilliant Blue, and Calcofluor White for screening of curli fimbriae and cellulose production (22℃, 4 d). The CDC Biofilm Reactor (CBR) was used with LB broth without salt for biofilm formation on stainless steel (22℃, 48 h). Upon harvest, the coupons were air-dried (22℃, 40 min) or acclimated under 33% RH (22℃, 4 d) for testing as a hydrated or desiccated biofilm, respectively. Treatment was carried out by submerging the coupons in oil (0.33 a_w) or 1% water-in-oil emulsion (0.92 a_w), at 60°C, with and without 200mM acetic acid. Biofilm matrix was observed with scanning electron microscopy.

Results: With the curli- and cellulose-binding assays, the colonies of *Salmonella* Enteritidis phage type 30 (ATCC BAA-1045) exhibited a red, dry and rough (rdar) surface morphotype associated with desiccation tolerance, emitted fluorescence under UV, and showed the greatest spatial distribution as compared to the other strains tested, thereby used for biofilm assays. After CBR growth, a bacterial concentration of 8.07±0.18 log CFU/coupon was obtained. Following desiccation, while the cell number decreased by 1.80±0.32 log CFU/coupon, the population's resistance to heat and acidified oil increased by a pronounced margin. Such enhancement, however, was offset by rehydrating the biofilm matrix or boosting the a_w of the heating menstruum. A 1-h treatment with acidified water-in-oil emulsion reduced hydrated and desiccated biofilms to levels undetectable by enrichment (TSB, 37℃, 24 h, n = 3x3).

Significance: Oil-based antimicrobials are effective against *Salmonella* biofilms.

T2-08 Insights into the FDA Food Facility Inspectional Violation Trends on Equipment and Environmental Sanitation: Key Learnings and Recommendations

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Introduction: Sanitary conditions of food equipment and the processing environment have been shown to influence the production of safe and quality food products within FDA-regulated facilities. Through historical trending of FDA Form 483 data from FYs 2006 to 2022, it was found that about one-third of inspectional observations have been directly related to at least 12 equipment and environmental sanitation issues. Industry and other stakeholders can use this information to gain insights and increase their food safety efforts.

Purpose: This study focused on FY 2023 FDA inspectional observations related to sanitation, whether there were any variances to the historical trends, and highlighted any key learnings the industry may benefit from.

Methods: Published data of FDA 483 Observations from FY 2023 was used, and the top 50 citations were refined to select those relevant to environmental and equipment sanitation. The results were then compared to key citations noted from FY 2006 – 2022.

Results: Citations with the highest frequencies were: 21 CFR117.35(a), 21 CFR117.35(c), and 21 CFR117.80(c). The top 5 issues of concern were: (1) failure to maintain the plant under sanitary conditions, (2) pest control, (3) inadequate precautions to control cross-contamination during processing operations, (4) unhygienic design or improper maintenance of equipment and utensils, and (5) facility hygienic design construction issues. FY 2023 citations did not seem to vary significantly from the historical observations and sanitation issues noted from FY 2006 – 2022.

Significance: The study reveals a systemic trend in inspectional violations over time. On an important note, the following industry-based recommendations should be required: (a) focusing on the controls, rather than risks, (a) appreciating the role of sanitation and other preventive controls for reducing inspectional violations, food recalls and foodborne outbreak occurrences, and (c) getting management buy-in to support an employee culture that drives food safety and sanitation system improvements.

T2-09 Rechargeable and Durable N-Halamine Surface Coating, Avantguard 247™, Treated with Chlorine-Based Sanitizer, Significantly Reduces Bacterial Load Long after Treatment to Enhance Food Safety Outcomes

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Introduction: Stainless steel is a commonly used material in the food processing industry. Sanitizing food contact and non-food contact surfaces is essential for preventing cross contamination from fomites to food through direct or indirect contact. In response to this need, AG247TM, an N-halamine surface coating was developed to extend and enhance the antimicrobial efficacy of chlorine-based sanitizers for over twenty-four hours.

Purpose: To evaluate the residual antimicrobial efficacy of diluted bleach-based sanitizer accompanied with N-halamine surface coating in a real-life

sanitizing routine in food processing environments.

Methods: An N-halamine surface coating was applied to vertical stainless-steel surfaces of a sausage cutting machine using the Biomist spray system. Sanitizer and cleaner were diluted per the product label, and then sprayed onto test surfaces on a daily basis. To better mimic real-world cleaning procedures, scrubbing with pads was included. Twenty-four hours after treatment with sanitizer, approximately six log (CFU/ 100 cm^2) of *Staphylococcus aureus* ATCC 6538 with 5% FBS or tripartite soil load was inoculated onto vertical stainless-steel surfaces. After drying for two hours at room temperature, residual *S. aureus* was harvested with 3M swabs, resuspended in letheen broth and plated onto 3M staph express Petri film for enumeration. Uncoated surfaces served as a negative control. Statistical analysis was performed using ANOVA.

Results: From non-coated surfaces, (3.56±0.32) log (CFU/ 100 cm^2) and (4.02±0.18) log (CFU/ 100 cm^2) of *S. aureus* was recovered in the presence of 5% FBS and tripartite soil load, respectively. From coated surfaces, (0.18±0.32) log (CFU/100 cm^2) and (0.00±0.00) log (CFU/100cm^2) of *S. aureus* in the presence of 5% FBS and tripartite soil, respectively.

Significance: Compared to using sanitizers alone, the inclusion of N-halamine coating in the cleaning routine can provide residual protection and significantly enhance antimicrobial efficacy outcomes from stainless steel surfaces (p<0.05) in the presence of soil loads.

T2-10 Safeguarding Herb and Spices Consumers in Europe: A Comprehensive Assessment of Chemical Hazard Identification

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❖ ***Developing Scientist Entrant***

Introduction: The increasing global interest in herbs and spices necessitates a thorough examination of the chemical hazards associated with their consumption. The European Rapid Alert System for Food and Feed (RASFF) has shown 1133 notifications for spices and herbs in the last ten years (2013-2023).

Purpose: This work was aimed at identifying the chemical hazards in herbs and spices and conducting a risk assessment based on the prevalence of these contaminants, focusing on mycotoxins, pesticide residues, and plant-derived compounds with potential health implications.

Methods: Comprehensive review of evidence in the public domain, using defined searching strategies (***e.g.***, Boolean operators and others) and data analysis.

Results: The analysis of RASFF notifications indicated that 58,7% (665) of the alerts corresponded to chemical hazards. Both literature data and RASFF showed that pepper (50) and oregano (39) were the products most contaminated. Of the known chemical hazards, chlorpyrifos (135) was most frequently detected in herbs and spices, followed by ethylene oxide (104), pyrrolizidine alkaloids (94), aflatoxin B1 (90) and ochratoxin A (39). Other contaminants included ochratoxin A, artificial unauthorized dyes, 2-chloroethanol, carbendazim, or polycyclic aromatic hydrocarbons in decrescent order of prevalence. The literature emphasizes the need to ensure the management of chemical hazards throughout the food supply chain, as consumers cannot mitigate these hazards during food preparation. Consequently, the analysis of spices and herbs is significant for quality control and human health safeguarding.

Significance: This study provides insight into the health risks associated with using herbs and spices and identifies data gaps needed for a more accurate risk assessment, fostering collaboration between all stakeholders to advance public health protection in Europe.

T2-11 The Most Appropriate Method of Verifying Absence of Sanitizer Contamination in Fluid Milk Depends on the Type of Sanitizer

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Introduction: There were several recorded incidents where milk contaminated with sanitizer was released into the market, indicating a clear need to re-evaluate and/or re-establish appropriate preventive controls by the fluid milk processors to prevent this type of adulteration of milk from occurring in the future.

Purpose: Identify common practices dairy processing facilities rely on to prevent contamination of fluid milk with different sanitizers and evaluate verification practices for effectiveness.

Methods: A survey was distributed via email to 11 fluid milk processors ranging in size and processing capacity. Based on the survey results, five separate verification practices were evaluated (i.e., sensory testing, rapid commercial test kits, pH, freezing-point depression, and ferric reducing antioxidant power (FRAP) assay) in relation to two different sanitizer types (i.e., sodium hypochlorite (NaClO), and peroxyacetic acid (PAA)). Sensory testing for the two separate sanitizer types was evaluated through three separate sensory experiments to determine (i) the sensory threshold limit, (ii) the effect of sensory training, and (iii) overall effectiveness of this common practice in a real dairy processing environment.

Results: Each fluid milk processor relies on up to 4 separate practices to assure absence of sanitizer contamination in milk. Only one of five evaluated practices per each of the two sanitizers showed potential to be used as verification method. Four separate commercial test kits were identified that can detect presence of PAA in milk at levels as low as 0.2 ppm. A smell test was identified as a method that may detect sodium hypochlorite sanitizer in milk at 20 ppm or lower if performed by panelists with appropriate sensitivity. A single sensory training session was determined to not provide relevant improvement in detection of sanitizers in milk.

Significance: Fluid milk processors must validate any practices they are using for verifying absence of sanitizer adulteration in fluid milk.

T2-12 UV-C Dry Sanitation of Visibly-Cleaned Salmonella-Inoculated Stainless Steel Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: There are no federal guidelines or regulations for equipment sanitation in low-moisture food processing environments, and pathogen persistence in these systems remains an ongoing industry challenge. Therefore, practical sanitation application parameters for visually clean stainless-steel surfaces are needed to improve food safety systems.

Purpose: The purpose of this study was to generate preliminary UV-C sanitation parameters for flour-soiled stainless-steel surfaces.

Methods: Stainless steel coupons (#304 mirror finish and brushed finish) were electrostatically coated with fabricated all-purpose flour containing a six-strain *Salmonella* cocktail (~7.8 log CFU/g) and immediately brushed to visibly clean. Thereafter, the stainless-steel coupons were exposed to UV-C (6 W, 254 nm) in a humidity-controlled chamber (45% RH) for 30 s at three distances (5, 10, and 15 cm) to evaluate the efficacy of UV-C for practical applications (including 0 s positive controls). Surviving *Salmonella* populations were recovered by hand-massaging bagged coupons with 5 ml of buffered peptone water (n=24). The sample was serially diluted, plated on modified Tryptic Soy Agar, incubated (48 ± 2 h, 37°C), and enumerated.

Results: Initial populations on visibly cleaned coupons (both #304 mirror finish and brushed finish) were 3.84 ± 0.15 log CFU/cm^2 (mean ± 95% CI). After 30 s, the average *Salmonella* populations were 1.79 ± 0.48 log CFU/cm^2 (5 cm), 2.23 ± 0.44 log CFU/cm^2 (10 cm), and 3.07 ± 0.48 log CFU/cm^2 (15 cm). Significant differences in populations were found for samples treated at 5 cm and 15 cm from the UVC emitter (p=0.002). Stainless steel finish (mirror or brushed) had no significant impact (α=0.05) on surviving *Salmonella* populations.

Significance: This study demonstrated the potential application of UV-C for dry sanitation on visually clean, unsanitized stainless steel surfaces.

T3-01 Benzalkonium Chloride Disinfectant Residues Stimulate Biofilm Formation and Increase Survival of *Vibrio* Bacterial Pathogens

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Introduction: *Vibrio* spp. are opportunistic human and animal pathogens found ubiquitously in marine environments. The global prediction of increasing ocean temperatures raises concerns about the heightened prevalence of *Vibrio* spp., impacting public health and the seafood industry. Effective strategies are urgently needed to control *Vibrio* spp. and prevent contamination, especially in aquaculture and seafood processing facilities.

Purpose: This study aimed to investigate the adaptation and survival of *Vibrio* spp. exposed to varying concentrations of benzalkonium chloride (BAC) residues, a common disinfectant used in these industries.

Methods: Experiments were conducted to assess the phenotypic adaptation of *Vibrio* bacteria, focusing on biofilm biomass changes. The study evaluated the strain-specific responses to BAC residues and their impact on physiological changes within *Vibrio* biofilms.

Results: Exposed *Vibrio* bacteria displayed phenotypic adaptation, characterized by an increase in biofilm biomass. The effect was found to be strain-specific rather than species-specific. BAC residue exposure induced physiological changes in Vibrio biofilms, resulting in an increased number of injured and alive cells within the biofilm. The study postulates that BAC may heighten the risk of viable, but non-culturable (VBNC) bacteria development, which poses a significant threat. VBNC bacteria, undetectable by standard culture-based methods, can lead to recurrent contamination events and disease outbreaks.

Significance: This research provides valuable insights into the role of c-di-GMP signaling pathways in *Vibrio* adaptation mechanisms, suggesting c-di-GMP mediated repression as a potential avenue for further investigation. The study emphasizes the potential risks associated with the misuse and overuse of BAC, highlighting the increased risk of biofilm development and bacterial survival within the seafood processing chain.

T3-02 Consumer-Focused Allergen Management: Going Above and Beyond to Protect Consumers

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Introduction: Allergen-related recalls continue to escalate globally, and consumer behavior towards allergen-containing products has changed as allergic population numbers rise. The food industry must continue to push for allergen control programs that are consumer-centric and go beyond what is required by local regulations.

Purpose: This project aimed to establish company-wide allergen management programs that maximize choice while minimizing risk based on our understanding of allergic consumer purchasing behavior, published scientific data, and manufacturing capabilities.

Methods: Between 2017 and 2019, surveys of allergic consumers were conducted (1700 participants) with European patient support groups to understand consumer behavior towards allergens and labeling which were then used to establish a systematic approach to allergen control. This approach was externally reviewed and determined to be industry leading.

Results: 37% of allergic consumers indicated that they look for cues in front of pack (e.g., product name, images, product description) that show all the allergens present in the product, 50% answered that they check labels every time they buy the product, and 35% responded that they only check the label if they buy the product for the 1st time. Given this, 2 industry-leading allergen programs were developed: 1) Product families: a group of products within a geographic region where the consumer would expect the same allergen profile. 2) Indicative levels: the maximum allergen carry-over level in a product with a Precautionary Allergen Labeling (PAL).

Significance: Understanding consumer behavior enables the development of allergen management programs that protect allergic consumers whilst providing maximum choice. We are actively working with regulators and industry groups to expand the adoption of these practices. These 2 programs were major contributory factors to Mondelēz winning the Black Pearl award in 2021.

T3-03 Detection of Cyclospora cayetanensis via Shotgun Metagenomics

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Introduction: Cyclospora cayetanensis infections are prevalent globally, and without the ability to culture this organism, additional molecular detection methods are critical for efforts to understand and control this food safety hazard.

Purpose: This work sought to evaluate the efficacy and optimize for the detection of *C.cayetanensis* via the use of shotgun metagenomics.

Methods: A lettuce sample that had been inoculated with 100 *C.cayetanensis* oocysts, as well as an uncontaminated control, were DNA extracted using Qiagen DNeasy PowerFood Microbial Kit, and the resulting community DNA was then sequenced at varying depths on both the Illumina MiSeq and Novaseq platforms. A sample of blueberries that had been naturally contaminated with *C.cayetanensis* was processed according to FDA BAM 19b, and the resulting DNA extraction was then subject to shotgun metagenomic sequencing on the Illumina MiSeq. Standard bioinformatic analysis was carried out using Kraken2 to assign taxonomy to sequence reads. Metagenome quality was assessed using MetaQUAST. Community composition impacts of different sequencing approaches were assessed via Shannon diversity index, and Bray-Curtis dissimilarity matrices.

Results: *C. cayetanensis* reads were detected in all inoculated lettuce sample sequencing runs. The inoculated lettuce sample that was sequenced at varying depths had a similar proportion of detected reads at all depths, suggesting substantial depth was not required for detection. The naturally contaminated blueberry sample was detected via shotgun metagenomics, with a higher proportion (0.347%) of detected reads than those obtained from spiked lettuce samples (~0.24%). Neither the standard Kraken database nor the version containing protozoa was sufficient for detection of *C. cayetanensis.* Either the construction of a custom database or alternative read mapping strategies was required for successful identification of *C. cayetanensis* reads in metagenomic samples.

Significance: This work demonstrates that shotgun metagenomics is a reliable method for the detection of C. cayetanensis in produce and informs on key factors to improve the design and execution of such detection.

T3-04 Disinfection Profiles of Common Disinfectants against Human Norovirus Using a Human Intestinal Enteroids Cultivation System

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Introduction: The human intestinal stem cell-derived enteroid (HIE) system's potential for assessing disinfectants against human norovirus (HuNoV) remains underexplored.

Purpose: We evaluated the effectiveness of common active ingredients of disinfectants (ethanol, isopropanol, and sodium hypochlorite) against HuNoV using HIE.

Methods*:* We deposited 10 µL of HuNoV suspension on stainless steel coupons to determine optimal drying times (0 to 120 minutes). We then tested disinfectants including sodium hypochlorite (5-1000 ppm) and alcohols (ethanol and isopropanol) ranging from 50% to 90% (v/v). Sodium thiosulfate (0.2%)

and fetal bovine serum (FBS) (1 to 10%) were used to neutralize sodium hypochlorite and alcohols. The neutralized disinfectants were tested for cytotoxicity on HIEs. Additionally, spin column (cellulose triacetate) was used to further purify the disinfectants to reduce chemical toxicity. In efficacy tests, 10 µL of norovirus suspension was placed on coupons, followed by a 15-minute drying period. Then, 50 µL of disinfectant was applied, and after one minute, 940 µL of each neutralizer was added. HIE monolayers were incubated with these preparations for 1 and 72 hours at 37°C and 5% CO_2. Viral reduction differences between test conditions were analyzed using the Student's t test, with significance at $P < 0.05$.

Results: Results showed that HuNoV retained viability for 25 minutes post-inoculation but lost > 2.1 log after 60 minutes. Sodium hypochlorite was non-toxic to HIEs, whereas neutralized alcohols were cytotoxic to HIEs, requiring additional removal using spin columns. Our findings suggests that persistence of HuNoV on surfaces was shorter than previously reported in studies using cultivable HuNoV surrogates. We also found that low concentrations of sodium hypochlorite (< 25 ppm) were ineffective against HuNoV dried onto surfaces.

Significance: Our data highlight the need for the use of effective neutralization methods for alcohols and common neutralizers, an important consideration for future norovirus disinfection studies using the HIE system.

T3-05 Fate of Common Foodborne Pathogens in Dark Green Leafy Vegetable Juice

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❖ ***Developing Scientist Entrant***

Introduction: Dark green leafy vegetable juices (DGLVJs) have received increasing attention due to their unique nutritional properties and taste. However, the more neutral pH associated with DGLVJs compared with fruit juices might put them in a higher microbial food safety risk category.

Purpose: The objective of this study was to determine the fate of common foodborne bacterial pathogens in DGLVJs and construct the primary and secondary model to predict their behaviors.

Methods: DGLVJs were prepared with commercial collard green, chard, and kale. The juices were inoculated with cocktails of rifampin-resistant *Listeria monocytogenes*, *Salmonella* spp., and *Escherichia. coli* O157:H7 at ca. 4.0 log CFU/mL and stored under refrigerated (4°C), ambient (23°C), and abused temperatures (37°C) for 21, 7, and 3 days respectively. The surviving pathogens were enumerated by plating onto selective agar and the data was used for establishing primary and secondary models with DMFit and Prism (Version 10.0).

Results: Baraniyi and Robert models showed a good fit (R^2>0.90) to describe the fate of pathogens in DGLVJs, except LM in chard and kale juice. A linear model was selected to describe the fate of *L. monocytogenes* in chard juice at 37°C (R^2=0.929) and kale juice at 4°C (R^2=0.721). Based on the secondary model, the changing rate of *L. monocytogenes*, *Salmonella* spp., and *E. coli* O157:H7 were -0.0014, -0.0004, -0.0022 log CFU/mL·h in collard green juice; -0.0014, -0.0011, and -0.0022 log CFU/mL·h in chard juice, -0.0015, -0.0010, and -0.0021 log CFU/mL·h in kale juice respectively.

Significance: By comparing with studies conducted for fruit juices, pathogens decreased slower and can survive longer in DGLVJs. The observation made from this study underscores the importance of implementing additional control strategies to ensure the microbial safety of DGLVJs.

T3-06 Food Safety Adherence and Bacterial Traits Linked to the Size of Food Establishments in Food Desert Regions of Central Virginia

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Introduction: Over 1M Virginians live in a food desert with low access to fresh, healthy foods. Fresh foods sold at small, independently owned corner markets and convenience stores (SIOMs) may have a high potential for reduced product quality and incur increased food safety code violations.

Purpose: Researchers assess market scale differences in food safety compliance and microbiological quality for select products procured from food deserts in Central Virginia.

Methods: From August 2018 to March 2020, 448 samples (fresh produce, meat, ethnic foods, food packaging surfaces) from 10 registered SIOMs and nine large chain supermarkets (LCSMs) were collected and analyzed.

Results: Researchers discovered significantly ($p<0.05$) higher levels of aerobic mesophile and coliform counts existed in SIOMs-acquired samples compared to LCSMs-acquired samples, indicative of SIOM lower food safety compliance rates. Surprisingly, *Campylobacter*, *E. coli*, *Listeria*, and *Salmonella* detection occurred in both SIOM and LCSM samples. Within SIOM samples, *Campylobacter* (76%, 16/21) and *Salmonella* (83.3%, 5/6) prevailed. Interestingly, SIOM samples exhibited lower multi-drug resistance (MDR) and non-susceptibility for *Campylobacter* and *Listeria* isolated samples compared to LCSM samples. Same-brand name commodities sold at SIOMs had significantly ($p<0.05$) higher prices than LCSM, which may indicate an increased financial burden for economically challenged residents in food desert areas.

Significance: Additional research to include a larger sample size is needed to validate the initial study findings further. Regardless of the scale of food outlets, increased food safety awareness and training are recommended to reduce risks of potential foodborne illnesses of vulnerable populations residing within food deserts.

T3-07 Linear and Non-Linear Inactivation Indices Associated with High-Pressure Processing and Thermally-Assisted High-Pressure Processing against *Listeria monocytogene*s

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a pathogen of public health concern that in more than 98% of cases are associated with consumption of contaminated food products. The pathogen is particularly of concern for ready-to-eat (RTE) commodities including cold-smoked fish products.

Purpose: Current study investigated the impact of treatments with elevated hydrostatic pressure for inactivation of *L. monocytogenes* on cold-smoked RTE trout at high and low inoculation levels.

Methods: Elevated hydrostatic pressure of 500 MPa were applied to RTE trout samples inoculated at target inoculations of 4.5 and 6.5 log CFU/g in a PULSE tube using a Hub880 barocycler unit. Temperature of the trials were set at 4.4 and 60.0°C, adjusted by a circulating water bath connected to stainless steel jacket surrounding the pressure processing chamber. The linear (D-value) and non-linear (K_{max}) inactivation indices were calculated for the challenge study and results were statistically analyzed using ANOVA followed by Tukey-adjusted means separation.

Results: Prior to pressure processing, the counts (selective counts of PALCAM, mean ± SD) of *L. monocytogenes* were 6.45 ± 0.1 log CFU/g and were reduced ($p<0.05$) to 3.72 ± 0.3, and <1.48 ± 0.8 log CFU/g after 10 minutes of treatment at 4.4 and 60.0 °C, respectively. Treatments of samples inoculated at low concentration of the pathogen were similarly efficacious and resulted in reduction ($p<0.05$) of the pathogen to 1.62 ± 0.3 and 0.82 ± 0.0 log CFU/g for treatments at 4.4 and 60.0 °C, respectively. At 4.4 °C, the linear D-value and non-linear k_{max1} were 8.68, and 0.50 and 5.81 and 2.41 for high inoculation and low inoculation samples, respectively.

Significance: Application of hydrostatic pressure at 500 MPa at cold and elevated temperatures were efficacious for up to 5.03 log CFU/g reduction of *L. monocytogenes,* illustrating the potential for further adaption of this technology in RTE aquaculture products.

T3-08 Photodynamic Inactivation of SARS-CoV-2 Surrogate Bacteriophage φ6 in Tomatoes Cross-Contaminated by Gloves

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Introduction: The SARS-CoV-2 pandemic increased the use of high-density polyethylene (HDPE) gloves for selection of fresh produce during shopping. Contaminated gloves may transfer viral particles transfer to food surfaces. Photodynamic inactivation (PDI) using blue light-emitting diode (LED) is proposed as a strategy with virucidal effects, but its efficacy against viruses on fruit surfaces is limited.

Purpose: This study analyzed the efficacy of PDI on inactivation of the SARS-CoV-2 surrogate bacteriophage φ6 on tomatoes surface cross-contaminated by HDPE plastic gloves.

Methods: Tomatoes cubes (2.5 x 2.5 x 0.2 cm; 3 ± 1 g) were cross contaminated (6.0 log PFU/sample) by pressing (30 s; 1.9 kPa) a gloved forefinger contaminated with φ6. Samples were immersed in a curcumin solution (75 μM; photosensitizer) for 2 min and exposed to PDI (LED at 430-470 nm wavelength and 33.6 mW/cm^2 of light irradiance; 2.01 J/cm^2 for min) for 8, 10 and 12 min. Before and after each exposure time, φ6 viral titer was determined using *Pseudomonas syringae* pathovar *phaseolicola* as host on Luria-Bertani agar (LB) incubated overnight at 25 °C, using the double-layer plaque assay. Treatments exposed to only LED and only the photosensitizer were evaluated as controls. Decimal reductions were calculated.

Results: After 8 and 10 min, PDI decreased the φ6 titer onto tomato surface by approximately 2.1 and 2.5 log PFU/sample, respectively. The reduction of φ6 titer increased (p<0.05) with increase of exposure time reaching γ of 2.9 log PFU/sample after 12 min. The φ6 titer did not change (p>0.05) on control sample surfaces.

Significance: PDI is promising as a technology for inactivating φ6 suggesting it may also be effective on SARS-CoV-2. The PDI efficacy appears time-dependent, and its use may help in minimizing spread of SARS-CoV-2 through cross-contaminated fruit surfaces.

T3-09 Prevalence and Carbapenem-Resistant *Escherichia coli* Pathovars from Fresh-Cut Fruits at Retail Outlets in Accra, Ghana

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Introduction: Globally, there is an emergence of carbapenem resistant enteropathogens in animal source foods, but little evidence exists in plant-based foods, especially those consumed raw.

Purpose: This study provides evidence of carbapenem-resistant *E. coli* and prevalence of *E. coli* pathovars in fresh-cut fruits vended in Greater Accra.

Methods: A total of 200 fresh-cut fruits sampled from retail outlets in 10 districts in Greater Accra consisting of pineapple (60), pawpaw (60), watermelon (60), and mix-fruits (20) were plated on Eosin Methylene Blue and Brilliance UTI agars. The antibiotic resistance profile of *E. coli* was determined by Kirby Bauer disc diffusion method for cefpodoxime, cefpodoxime/clavulanic acid, meropenem, trimethoprim/sulfamethoxazole and pefloxacin on Mueller Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) standards. Multiplex PCR was used to identify *E. coli* virulence genes (*est, elt, stx1, stx2, eae, aggR, pic, invE, ipaH, ent, escV),* Extended Spectrum Beta-Lactase (ESBL) inhibitor genes (*blaSHV, blaTEM, blaCTX-M),* and carbapenemase genes (*blaIMP, blaKPC, blaOXA-24, blaSPM*).

Results: The overall prevalence of *E. coli* was 17%, while prevalence on the cut fruits were pineapple: 6.7% (4/60), pawpaw: 16.7% (10/60), watermelon: 25% (15/60), and mix-fruit: 25% (5/20). All 34 *E. coli* isolates harbored virulence genes associated with STEC, EIEC and EPEC, most of which were hybrid strains: *stx1*(97%), *stx2*(100%), *ipaH*(94%), *escV*(32%). Among the 34 *E. coli* isolates, phenotypic resistance was observed for ESBL antibiotics (44.1%), cefpodoxime (20.6%) and meropenem (5.9%) indicating broad resistance to beta-lactam group of antibiotics, including cephalosporin and carbapenem resistance. The genes associated with phenotypic antibiotic resistance were $bla_{CTX\text{-}M}$(for ESBLs) and bla_{IMP} (for carbapenemase).

Significance: The presence of hypervirulent carbapenem and ESBL resistant hybrid *E. coli* pathovars in fresh-cut fruits suggests a potential public health risk, particularly to immunocompromised consumers, as last resort carbapenem antibiotics may be ineffective in treating *E. coli* infections.

T3-10 Quantitation of Barley and Rye Gluten in Select Fermented Dairy Products by a Multiplex-Competitive ELISA

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Introduction: Accurate quantitation of the gluten in fermented or hydrolyzed foods is a challenge primarily due to variable proteolysis and the lack of appropriate calibrants. Addressing the variability in gluten quantitation from wheat, rye, and barley is another inherent challenge of all gluten detection methods.

Purpose: A multiplex-competitive ELISA was previously single laboratory validated for the quantitation of wheat gluten in fermented yogurt, kefir, and buttermilk. In this study, we evaluated the ability of the ELISA to quantitate barley and rye gluten in these matrices.

Methods: Yogurt, kefir, and buttermilk were prepared by incurring (before fermentation) 0, 8, 20, and 100 µg/mL of barley and rye gluten, and then analyzed by multiplex-competitive ELISA**.** Barley and rye gluten-spiked (at 0 and 20 µg/mL gluten) samples were also prepared and analyzed. The 20 µg/mL barley and rye gluten incurred samples were further analyzed using commercial RIDASCREEN Gliadin Competitive ELISA. Statistical validation parameters including limit of detection (LOD), limit of quantitation (LOQ), % recovery and % coefficient of variation (CV) were determined.

Results: The LOD and LOQ of the method were 1.2 and 3.0 µg/mL, respectively, for the quantitation of barley gluten. For rye gluten, the values were 1.3 and 2.6 µg/mL, respectively. Average gluten recoveries from the incurred and spiked yogurt, kefir, and buttermilk were 54-215% and 55-250% for barley and rye, respectively. Only a few samples exceeded 200% recovery. The % CV was less than 20% for most of the samples. The multiplex-competitive ELISA showed greater accuracy in quantitating barley and rye gluten compared to the RIDASCREEN Gliadin Competitive ELISA, which resulted in >400% recovery for many samples.

Significance: By applying alternative quantitation strategies, the multiplex-competitive ELISA can provide accurate and precise quantitation of barley and rye gluten, in addition to wheat gluten, in select fermented dairy products.

T3-11 Ready-to-Eat Foods as Human Exposure Matrix for Pathogenic *Vibrio* Species: A Retrospective Observational Study

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Introduction: The global increase in foodborne infections has become a public health concern with a major contribution from ready-to-eat foods (RTEFs) due to various reasons.

Purpose: The present study aimed at assessing the prevalence of *Vibrio* in RTEFs.

Methods: *Vibrio*-RTEFs data were mined from four repositories (made of large consortium) using topic-specific algorithm "vibrio AND ready-to-eat" from 1999–2023 and fitted to a random intercept logistic regression in common-effects and mixed-effects group analytic models. The model's robustness was cross-validated via leave-one-out analysis.

Results: Global prevalence of *Vibrio* in RTEFs was 16.97% (95%CI: 16.33–17.63), with a prediction interval (PI) of 0.01–88.35%. The cross-validation yielded

11.51% (9.26–14.21; PI: 8.65–15.16). WHO regional prevalence of *Vibrio in* RTEFs was significantly different (p<0.01) with the highest from Africa (9.74%, 9.12–10.39), South-East-Asia (8.32%, 0.64–56.17), Western Pacific (1.34%, 0.27–6.42), European (0.26,0.00–12.76), and Eastern-Mediterranean (0.23%, 0.00–100.0) regions. Continent-based prevalence of *Vibrio in* RTEFs was significantly different (p = 0.04) with the highest from Africa (12.75%, 1.66–55.86), then Asia (1.72%, 0.48–5.91) and Europe (0.03%, 0.00–95.56). *Vibrio* was more prevalent in RTEFs of plant (8.57%, 1.99–30.19) compared to animal (4.33%, 0.82–19.84) and mixed (plant and animal) (0.34%, 0.04–2.67) origins. *V. mimicus* (10.73%, 5.94–18.61) was the most prevalent, then *V. fluvialis* (6.62%, 0.07–86.80), *V. harveyi* (8.91%, 0.00–100.0), *V. alginolyticus* (5.46%, 0.09–78.28), *V. parahaemolyticus* (7.15%, 3.75-13.23), *V. cholerae* (3.18%, 0.48–18.35) and *V. vulnificus* (1.70%, 0.34–8.16). *tdh* (11.72%, 1.40–55.36) and *trh* (11.21%, 0.90–63.69) of *V. parahaemolyticus* are the most tested virulence genes.

Significance: *Vibrio* pathogens in RTEFs pose health risk and significantly endanger public health sustainability and are time-bomb for health emergencies. Appropriate implementation of standard hygienic procedures in the production line of RTEFs is paramount to prevent human exposure and subsequent health emergencies.

T3-12 Utilizing Zebrafish Embryos for Replication of Tulane Virus – A Human Norovirus Surrogate

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❖ *Developing Scientist Entrant*

Introduction: Zebrafish larvae and embryo models for replication of certain strains of human norovirus (HuNoV)—a leading global cause of viral gastroenteritis—have been recently reported. However, before the discovery of the zebrafish and human intestinal enteroid models to study HuNoV, surrogate viruses, such as Tulane virus (TuV), were used for research. As acquiring a sufficient amount of HuNoV for research remains challenging, identifying surrogate viruses capable of replicating in the zebrafish embryo model holds significant value.

Purpose*:* To determine if zebrafish embryos support TuV replication and thereby explore further utilities of TuV as a surrogate for HuNoV using the zebrafish model.

Method: Fertilized zebrafish eggs were microinjected with 3 nl of TuV at a concentration of 9 log RNA copies/mL within 4 hours post-fertilization and maintained at 32°C in petri plates containing Danieau's solution. Each day post-infection (dpi), 10 embryos were pooled as a single sample, and two samples were collected daily until 5 dpi. RNA was extracted from the harvested embryos and quantified using droplet digital PCR. Three experimental trials were conducted, and the data were analyzed in R using a linear model. Group means and their corresponding 95% confidence intervals were calculated using estimated marginal means. Multiple pairwise comparisons were performed to identify any statistical differences.

Results: A significant increase in TuV RNA copies, exceeding 5 logs per embryo, was observed at 2 dpi. The highest levels of the virus were detected at 4 dpi (6.22 log RNA copies/embryo). On the last day tested, at 5 dpi, the viral RNA levels declined to below 6 logs, similar to the levels observed at 3 dpi.

Significance: The replication of TuV in zebrafish embryos indicates a similar pathogenesis to that of HuNoV, thereby validating its use as a dependable surrogate for HuNoV.

T4-01 *Salmonella* Prevalence and Serovar Populations in Surface Waters are Driven by Proximal Land Use

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Introduction: It is critical to understand the factors that drive foodborne pathogen transmission in freshwater environments.

Purpose: This study was designed to determine the incidence of multiserovar populations in creek water, as well as to evaluate the impact of environmental factors, such as weather and surrounding land use, upon *Salmonella* prevalence and population complexity.

Methods*:* Over 24 months (November 2021-October 2023), 10L water samples were collected from 19 sites across four creeks (n=456). Each creek was defined by distinct surrounding land-use, including animal agriculture (two creeks), suburban development, and a national forest. Meteorological data was recorded from nearby weather stations. In addition to *Salmonella* isolation, deep serotyping was performed to characterize *Salmonella* serovar populations.

Results*:* Overall *Salmonella* prevalence was 66.4% (303/456) and differed based on the creek and season (Chi squared, p=0.046 and p<0.001, respectively). Prevalence was highest during spring (93.0%, 106/114) and lowest in summer (37.7%, 43/114). Deep serotyping of positive samples from the first 14 months identified 25 serovars in total and revealed that 85.7% (132/154) samples contained multiple serovars, (range 1-8 serovars/sample), with an average of 3.3 serovars/sample. The incidence of multiserovar populations was highest in spring (3.9 serovars/sample) and ranged from 91.2% in two different creeks (national forest, 31/34; animal agriculture, 52/57) to 77.3% in the suburban creek (17/22). Generalized linear mixed-models identified wind speed, humidity, and the ratio of herbaceous land to wetland and pasture as factors that drive *Salmonella* prevalence in these systems. Contrary to previous studies, *Salmonella* prevalence did not increase following precipitation.

Significance: These data from four different creek systems show that *Salmonella* prevalence and population complexity in freshwater can be driven by weather, season, and proximal land-use. This information can be used to better predict *Salmonella* risk in surface water based on particular environmental and meteorological circumstances.

T4-02 Targeted Mass Spectrometry Method for Detection and Quantification of Total Egg Protein from Multiple Processed Food Matrices

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❖ *Developing Scientist Entrant*

Introduction: Egg is a priority food allergen worldwide. Accurate and reliable detection plays a pivotal role in allergen management. Mass spectrometry (MS) has the potential to accurately quantify egg protein in processed foods.

Purpose: This study aimed to evaluate a targeted MS method for quantifying egg proteins in four processed food matrices (cookies, pie crust, pasta, and ice cream).

Methods: Ten egg-specific target peptides were identified from whole egg powder (WEP) and used to develop an MS method for quantification of egg protein in four food matrices, with a calibration range of 0.25 to 100 ppm WEP. The performance of the MS method was evaluated by examining the probability of detection (POD) across eight calibration curves and quantifying egg protein from WEP-incurred food matrices (n=6, 1, 2.5, 5, 10, and 100 ppm WEP). Additionally, the performance of the MS method was compared with that of commercial egg ELISA kits using the same WEP-incurred food matrices.

Results: In assessing the sensitivity of the MS method, three of the 10 target peptides were detectable at 2.5 ppm WEP across all calibration curves (POD=1, n=16). All 10 target peptides were quantifiable in the 100 ppm WEP matrices, and one peptide was quantifiable across all 1 ppm WEP matrices. The detection of egg protein in the food matrices was significantly improved by the MS method compared to the ELISA kits (one-way ANOVA with Tukey's test, p<0.05). Recoveries from the 10 ppm WEP foods using the MS method were 61% in cookies (with a maximum recovery of 39% among the ELISA kits), 76% (33%) in pie crust, 61% (52%) in pasta, and 94% (79%) in ice cream.

Significance: The MS method was sensitive and could reliably detect and quantify 1.2 ppm total egg protein in four incurred food matrices with >60% egg protein recovery.

T4-03 Investigation of Biofilms of *Salmonella* and *Listeria* Along with Dominant Genera of Retail Environments

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Introduction: Foodborne pathogens form robust biofilms with surface microbiota, presenting challenges due to heightened sanitizer resistance.
Purpose: This study explores interactions among bacteria derived from retail environments (*Serratia liquefaciens* and *Pseudomonas simiae*) and foodborne pathogens (*Salmonella* Typhimurium and *Listeria monocytogenes*) within biofilms, specifically examining how biofilm compositions shield pathogens from quaternary ammonium-based sanitizer (QUAT).
Methods: For each replication, mono, binary, and ternary biofilm structures (totaling 10) were studied. Binary biofilms consisted of one retail-derived bacterial species paired with a foodborne pathogen, while ternary biofilms included both retail-derived bacteria alongside a foodborne pathogen. Mono-biofilms were developed with a bacterial inoculum concentration of 6 log CFU/mL. Mixed biofilms were developed using a cocktail containing an equal ratio of the respective bacterial inoculums. Biofilms were grown for 24 hours at 25 °C in 96-well polystyrene microtiter plates, using 200 µL bacterial cocktail aliquots. Following biofilm formation, one set served as a control, while others were treated with 50 PPM or 200 PPM QUAT for 2 minutes. Biofilms were then analyzed for bacterial counts (log CFU/well). The experiment replicated three times, and statistical analyses were conducted using one-way ANOVA and Tukey's test.
Results: In mono-biofilms, *Serratia* demonstrated the highest biofilm formation ability, while *Listeria* exhibited the lowest (p=0.03). However, such variation was absent in mixed biofilms. After 50 PPM QUAT treatment, consistent reductions were observed within mono, binary, and ternary biofilm structures. *Serratia* showed reductions ranging from 0.59 to 2.4, *Pseudomonas* 1.29 to 2.7, *Salmonella* 0.78 to 1.64, and *Listeria* 1.67 to 3.34. Following 200 PPM QUAT treatment, reductions were significantly different only within mono-biofilms (p=0.01), with *Listeria* (4.33) experiencing the highest reduction and *Salmonella* (0.79) the least.
Significance: This study emphasizes the need for better biofilm management practices due to bacteria's diverse biofilm-forming capacities, inter-species interactions, and sanitizer resistance.

T4-04 Effect of Organic and Conventional Fertilizers in the Survival and Biofilm Formation of *Salmonella* in Irrigation Distribution Systems

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Introduction: Biofilms in water distribution systems such as drip or drop hoses can play an essential role in spreading pathogens into downstream irrigated crops and thus should be evaluated as a potential harborage point.
Purpose: In this study, we evaluated the formation of *Salmonella* biofilms in irrigation line in the presence of conventional (C) or organic (O) fertilizers in the lab setting.
Methods: Drip tubes (without emitters) were filled with 100 mL of either pond water (PW), PW with 1% (*v/v*) conventional (C; 4-0-8), or PW with 0.1% (*v/v*) organic (O; 2-4-1) fertilizer (N=108). Filled water was inoculated with 1 log CFU/mL population of a Rifampicin-resistant *Salmonella* cocktail, and tubes were incubated at 21.1°C for 21 days. Water was replaced with non-inoculated PW on days 7, 10, 14, 17, and 21 to mimic irrigation events. *Salmonella* population in the water and attached to the tubing was determined on days 0, 7, 14, and 21 [limit of detection (LOD): 0.15 log CFU/mL] along with Scanning Electron Microscopy (SEM).
Results: From day 0 to 7, *Salmonella* populations in water significantly increased by 3 log CFU/mL in O (p=0.0023), remained constant in PW, and decreased below the LOD in C. Populations on the O tubes significantly increased by 6 log CFU/mL from day 0 to 7 (p=0.016) and remained constant until day 21. For PW, populations in tubes significantly increased by 3 log CFU/mL (p=0.02) and then decreased until day 21. Populations remained below the LOD for C for all sampling events. Cross-contamination from the tube to the non-inoculated water for the O and PW treatments was observed. Biofilm formation was evident in O and PW treatments from SEM.
Significance: This work has established that fertilizers and contaminated irrigation water can affect the formation of *Salmonella* biofilms and, in certain instances, be a vector for cross-contamination.

T4-05 Food Nano-Safety: Harnessing of Green-Synthesized Nanoparticles for Enhanced Antimicrobial Action against *Campylobacter jejuni*

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Introduction: *Campylobacter jejuni*, a foodborne bacterial pathogen, poses a significant global health threat, especially through poultry-related infections due to cross-contamination and inadequate cooking practices. Conventional antimicrobial methods face challenges in combating *C. jejuni*, necessitating innovative strategies for inhibiting the growth of this pathogen.
Purpose: The study aimed to investigate the antimicrobial activity of metallic nanoparticles (NPs) synthesized through green synthesis against *C. jejuni*, proposing a novel strategy with potential applications for the poultry industry.
Method: Green synthesis of stable copper oxide nanoparticles (CuONPs) and silver nanoparticles (AgNPs) was conducted using intracellular components of the medicinal fungus *Ganoderma sessile*. Characterization of the NPs' synthesis employed transmission electron microscopy analysis, dynamic light scattering, and Zeta potential measurement. The growth inhibition of *C. jejuni* was determined by quantifying colony-forming units on anaerobe basal agar under microaerophilic conditions in the presence of different concentrations of the NPs.
Results: The successful green synthesis approach demonstrated effective growth inhibition of *C. jejuni* by NPs, impacting bacterial cell structures. Practical applications were highlighted by determining the minimal inhibitory concentration (MIC) values of CuONPs at 10 µg/ml and AgNPs at 6.7 µg/ml, as confirmed by performing *in vitro* tests to assess *C. jejuni* growth and survival. Further characterization data showed quasi-spherical nanoparticles embedded in an organic matrix, and these analyses revealed stable CuONPs with sizes of 2.9 ± 0.9 nm and a zeta potential of -21.0 mV. AgNPs exhibited a size of 14.7 ± 0.6 nm and a zeta potential of -24.4 mV, ensuring stability. Ultra-structure assays further elucidated the NP's interaction with the outer membrane of *C. jejuni*.
Significance: This research presented the first demonstration of using green synthesis, specifically with the medicinal mushroom *Ganoderma*, to produce nanoparticles that effectively inhibit *C. jejuni* growth, providing a sustainable and alternative approach to the traditional use of antibiotics.

T4-06 Impacts of Relative Humidity on Inactivation of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 on Dried Basil Leaves by Gaseous Ozone

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❖ *Developing Scientist Entrant*

Introduction: Amidst rising *Salmonella*-associated outbreaks of spices, there is a pressing need to explore non-thermal gaseous technologies that effectively inactivate pathogens while preserving their quality.

Purpose: The goal of this study was to determine how relative humidity affects the efficacy of gaseous ozone in inactivating *Salmonella* on dried basil leaves, and to explore the surrogate suitability of *Enterococcus faecium* for this treatment.

Methods: Dried basil leaves were inoculated with cocktails of *Salmonella enterica* (Montevideo, Mbandaka, Agona, Reading, and Tennessee) and *E. faecium* and equilibrated to water activity 0.55. Two grams of inoculated samples were then treated at 900-930 ppm ozone concentrations at different relative humidity levels (70, 80, and 90%) over 1-5 h. Two primary models (Log-linear and Weibull) and a secondary Modified Bigelow model were used to fit the inactivation data of both bacteria. Quality analysis was conducted post-gaseous ozone treatment. Means of inactivation and quality data from 3 batches were compared with JMP Pro (α=5%).

Results: The combination of 900-930 ppm gaseous ozone concentration and 90% RH over 5 h provided reductions of 5.3 ± 0.4 and 4.6 ± 0.2 log CFU/g for *Salmonella* and *E. faecium*, which were significantly greater than those of 70 and 80% RH (1.18-1.55 and 1.68-1.94 log CFU/g for *Salmonella* and *E. faecium, p*<0.05). Increased resistance of *E. faecium* compared to that of *Salmonella* indicated its suitability as a surrogate on ozone-treated dried basil leaves at 90% RH. The log-linear model fitted the data best when compared to the Weibull model parameters (lower RMSE and AICc). Gaseous ozone treatments did not significantly impact the color, antioxidants, or total phenolics (*p*<0.05).

Significance: These findings can serve as a useful reference to the spice industry for developing and validating the most effective gaseous ozone treatments to inactivate pathogens while preserving spice quality.

T4-07 Most of the Salmonellosis Risk in Raw Chicken Parts is Concentrated in Small Amounts of High Levels and High Levels of High-Virulent Serotypes of *Salmonella*

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❖ *Developing Scientist Entrant*

Introduction: *Salmonella* prevalence has reduced in U.S. raw poultry products since adopting prevalence-based *Salmonella* performance standards, but human illnesses did not reduce proportionally.

Purpose: To determine better indicators of risk, we used QMRA to evaluate public health risks of raw chicken parts contaminated with different levels of (1) all *Salmonella* and (2) high- or low-virulent *Salmonella* serotypes.

Methods: *Salmonella* level distributions were fitted to Lognormal distribution using data from the 2012 USDA-FSIS Baseline Survey ("Baseline") and 2023 USDA-FSIS routine verification sampling ("HACCP"). Three different Dose-Response(DR) models were used to explore the public health risk of *Salmonella* levels and serotypes:(1)Single DR for all serotypes; (2)Reduced virulence for Kentucky; (3)Multiple serotype-specific DR models.

Results: All QMRAs indicated concentrated risk in relatively small proportion of products contaminated with high levels of *Salmonella*. Our simplest approach using a single DR model with Baseline data(μ=-3.19,σ=1.29) showed 68% and 37% of illnesses were attributed to 0.7% of products above 1CFU/g and 0.06% of servings above 10CFU/g, respectively. More recent HACCP data(μ=-4.85,σ=2.44) showed 99.9% and 99.6% of illnesses were attributed to 2.3% of servings above 1CFU/g and 0.8% of servings above 10CFU/g, respectively. The QMRA with serotype-specific DR models showed more concentrated risk than the simpler approaches at all levels. Baseline data showed 91.5% and 63.7% and HACCP data showed >99.9% and 99.9% of illnesses were attributed to products above 1 and 10CFU/g, respectively. Regarding serotypes, 0.003% and 0.3% of illnesses were attributed to 0.2% and 0.7% of products above 1CFU/g of Kentucky while 69% and 78.7% of illnesses were attributed to 0.3% and 1.2% of products above 1CFU/g containing Typhimurium, Enteritidis, or Infantis for Baseline and HACCP data, respectively.

Significance: Our QMRA suggests public health risk is concentrated in a small proportion of products contaminated with high levels and specifically high levels of high-virulent serotypes. Low-virulent serotypes, such as Kentucky are predicted to contribute to extremely few human cases.

T4-08 Utilizing 16S Sequencing with Viability Differentiation to Identify Potential Sources of Spoilage Contamination During RTE Meat Manufacturing

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Introduction: Microbial biomapping has become a valuable tool for the meat and poultry industry. Despite its growing popularity, even the more advanced molecular-based approaches are still limited by their inability to differentiate based on viability.

Purpose: The objective of this study was to utilize a combination of molecular techniques to generate a microbial biomap of the viable population to identify potential sources of contamination and microbial shifts that occur during the manufacturing of a commercial RTE meat product.

Methods: Triplicate samples were collected at the following locations a total of nine times: (A) raw, (B) after oven, (C) after chiller, (D) holding cooler, (E) staging, (F) after slicer, (G) packaged product (N=189). Portions of each sample (25 g) were homogenized in PBS (25 mL) and centrifuged. The resulting pellets were resuspended in PBS and treated with PMAxx solution (25 μM) to remove non-viable DNA. The remaining DNA was extracted using Qiagen DNeasy Blood & Tissue Kit, sequenced on an Illumina MiSeq, and analyzed in QIIME2. Statistical significance of α and β-diversity metrics were determined using ANOVA and ADONIS, respectively, and ANCOM was performed to identify differentially abundant taxa. Main effects were considered significant at *p*≤0.05 and pairwise at Q≤0.05.

Results: Location had a significant impact on both the richness and evenness of the microbial population. Raw samples possessed the least rich and most uneven population with an increase in both metrics observed after thermal processing. Microbial shifts were observed after cooking and slicing. At the genus level, 11 taxonomic groups were identified as differentially abundant by location. The most noteworthy being *Lactobacillus*, a common spoilage organism, which was found in greater abundance after slicing.

Significance: This study demonstrates the utility of viable microbial biomapping as a diagnostic tool to identify potential sources of contamination in an RTE manufacturing process.

T4-09 Dynamics of Change in Physiological State of *Escherichia coli* O157:H7 during Cold Storage of Romaine Lettuce

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Introduction: *E. coli* O157:H7 can contaminate and survive on leafy greens through distribution chain. Once harvested, lettuce is cooled and can then be processed, or transported refrigerated for days before Cold stress exposure can affect bacterial physiology, inducing entry into dormant states like persisters and viable but non-culturable (VBNC).

Purpose: Determine effect of harvest temperature and cold storage length on *E. coli* O157:H7 physiological state.

Methods: Greenhouse-grown Romaine lettuce, sprayed with three *E. coli* O157:H7 outbreak strains separately, was harvested after 24h and stored at 2°C for 5d following 4h at harvest temperature (17°C or 9°C). Lettuce samples, collected over 5d, were homogenized, plated, exposed to 10X MIC of ciprofloxacin for persisters and subjected to PMA-qPCR for VBNC. Statistical analysis included correlation tests, ANCOVA to test effect of harvest temperature on strain over time and regression tree analysis to compare effect of harvest and cold storage together over time.

Result: Over 5d of cold storage, persister formation was positively-correlated (r=0.14, p=0.022) and VBNC formation had a weak negative-correlation (r=-0.11, p=0.065) for lettuce harvested at 17°C. Persister (r=0.09, p=0.441) and VBNC formation (r=0.02, p=0.800) were not significantly correlated with time for lettuce harvested at 9°C. The interaction between harvest temperature and strain was significant for formation of VBNCs (df=2, p<0.001). Highest average increase in VBNCs was found in the Central Coast 2018 outbreak strain (1.7 log CFU/g) for lettuce harvested at 9°C. The interaction between strain and harvest temperature affected the persister fraction (df=2, p=0.18). Central Coast and Salinas outbreak strains on lettuce harvested at 9°C and stored for >12 hours had the highest (37%) persister formation.

Significance: Changes in E. coli O157:H7 physiology on lettuce during cold storage will be integrated into a QMRA-based tool for producers, which will assist in identifying practices that minimize the risk of E. coli O157:H7 transmission through lettuce.

T4-10 Mechanistic Insights into the Role of α/β-Type Small Acid Soluble Protein and Inner Membrane Proteins during Bacterial Spore Inactivation by Ohmic Heating

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Introduction: It is established that Ohmic heating (OH) offers higher inactivation of spores compared with conventional heating (CH). But the mechanism behind this increased killing remains unknown.

Purpose: The study aims to find how electrical component of OH increases spore killing.

Methods: Genetically modified spores without essential components were utilized to investigate the interaction between spores and the electrical effects of OH. By comparing inactivation outcomes with standard spores, this helps identify the components affected by OH's electrical aspect, shedding light on the mechanism of spore elimination. The study examines the impact of electric field strength on *Bacillus subtilis* PS533 (wild type), PS578 (lacking small acid-soluble proteins), PS4461 & PS4462 (has 2Duf protein in the spore's inner membrane, affecting permeability and mobility), comparing results with CH. Experiments involved three field strengths (30, 40, and 50 V/cm) and three final temperature settings (95, 105, and 115°C).

Results: The findings reveal a strong impact of field strength on spore inactivation, showcasing that higher field strengths at specific temperatures result in increased inactivation rates. For instance, at 115 ºC, the inactivation of *B. subtilis* PS4461 (wild-type) increased from 1.10±0.03 to 5.20±0.99 CFU/ml as the field strength increased from 30 to 50 V/cm. Additionally, heat alone demonstrated lower efficacy in spore elimination. At 115ºC, OH exhibited nearly double the inactivation of conventional heating under the same conditions (1.10±0.03 CFU/ml versus 0.40±0.09 CFU/ml for CH) for wild type spore. Moreover, OH and CH inactivation profiles closely aligned when SASP and 2Duf were absent, suggesting that these components are influenced by the electrical aspect of OH. Furthermore, significant killing of spores was at lower temperatures was only observed for 50V/cm, suggesting some effects of OH only active at higher applied field.

Significance: The study's results will assist in optimizing the OH process to rapidly eliminate bacterial spores, ultimately enhancing the quality of food products.

T4-11 Zero-Inflated Negative Binomial Modeling to Assess of Generic *E. coli* Presence in Soil Amended with Untreated Manure in Certified Organic Farms

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Introduction: Generic *E. coli* (gEc), an indicator for foodborne pathogens in soil, is frequently tested negative, which challenges the assumption of normality in linear regression when modelling the risk factors. In this study, a zero-inflated negative binomial model was adopted to better address the excessive zero values of gEc to investigate the risk factors influencing the gEc in soil obtained from USDA-National Organic Program (NOP) certified organic farms with a mathematically favored model.

Purpose: To identify the risk factors associated with gEc in untreated manure-amended soil from organic farms across four regions in the US.

Methods: USDA-NOP certified organic farms (n=19) representing California, Minnesota, Maine, and Maryland were recruited in this two-year (2017-2018) study. Soil samples were collected during the two growing seasons and analyzed by demographic information, irrigation water, pathogen, gEc presence, and certain soil characteristics. A zero-inflated negative binomial regression with gEc baseline data was employed to assess the risk factors of the up-rounded log-transformed Most Probable Number (logMPN) of gEc in the soil.

Results: Among all samples across the four states, 39.94% (961/2406) of soil samples were gEc-negative and the logMPN ranged from 1 to 7. Samples collected after Day 30 post manure application had a lowered risk of gEc counts. Application of poultry-manure (RR=2.48, p<0.001) and presence of *Listeria monocytogenes* in soil before manure application (RR=1.38, p=0.001) significantly increased the risk of gEc contamination in soil samples. In contrast, presence of *Salmonella* in soil before manure application (RR=0.182, p<0.001) and presence of gEc in irrigation water (RR=0.317, p=0.005) significantly decreased the risk of logMPN of gEc in soil.

Significance: This study indicates potential risk factors associated with fecal bacterial survival in pre-harvest fresh produce cropping environments in soils amended with raw manure on NOP-certified organic farms.

T4-12 Risk Ranking of Antibiotic-Resistance Genes on Human Health

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❖ *Developing Scientist Entrant*

Introduction: Antimicrobial use in agricultural settings has exacerbated the threat of antibiotic resistance to human health over recent decades. However, not all antibiotic-resistant genes (ARGs) pose an equal risk.

Purpose: Establish a ranking system for known ARGs which will subsequently be used to assess human health risks associated with agricultural watersheds in Nebraska and Iowa.

Methods: In the initial stage of the study, Human Accessibility Potential Index (HAPI) and pathogenicity were investigated. A total of 52,515 high-quality metagenome-assembled genomes covering various habitats were included from the literature. HAPI was defined based on gene presence across diverse environments and specificity to the human microbiome. K-means clustering with an optimized number of k, was utilized to categorize genes based on HAPI values. To assess pathogenicity, all bacterial RefSeq genomes were screened for ARG annotation. The pathogenicity of genes was quantified as the odds of their presence in ESKAPE pathogens compared to non-pathogens (i.e., odds ratio).

Results: A total of 1,176 ARGs were grouped based on the HAPI value. Around 29% of ARGs were in high (1.28<HAPI≤1.85) and very high (1.85<HAPI≤2.47) categories, 35% in the moderate (1<HAPI≤1.28), and the rest clustered as low (0<HAPI ≤1) and very low (HAPI = 0). Regarding pathogenicity, 59% of investigated ARGs originate from pathogens. Among them, most were unique to one pathogen, while 50 were shared between four pathogens, and 2 genes (*qacJ, qacG*) responsible for multidrug resistance were shared between five pathogens. Fifty-six percent of the ARGs had an odd ratio of greater than one indicating a positive association with being present in pathogens. Overall, *evgA, emrK*, *emrY* were the top-ranked ARGs demonstrating both high HAPI and pathogenicity.

Significance: Our approach represents next-generation risk assessment by incorporating genomic data, which may facilitate more effective surveillance of quantifiable health risks, resonating with the "One Health" initiative.

T5-01 Host Adaptation and Niche Specialization of Pathogenic *Escherichia coli*

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Introduction: Pathogroups of *Escherichia coli* are defined by specific virulence factors. Some pathogroups are highly associated with serotypes, such as Shiga-toxin producing *E. coli* (STEC) and *E. coli* O157:H7. Specialization of adhesion factors for colonization and stress resistance genes may provide indications of adaptation to specific hosts or environments.

Purpose: The study aimed to investigate the relationship among phylogeny, serotypes, and the presence of virulence factors and stress/antimicrobial resistance genes (SR/AMR) using a large set of *E. coli* genomes.

Methods: Over 13,000 *E. coli* genomes were retrieved from GenBank, in *silico* subtyped for serotypes and phylogroups, and screened for genes coding for virulence factors and SR/AMR based on the Virulence Factor Database. The analysis focused on high-quality genomes representing non-redundant serotypes to discern associations of toxin, adhesion, and SR/AMR using Pearson's $\chi 2$ test ($p<5\times10^{-6}$).

Results: Many but not all of the 1,702 serotypes of *E. coli* are monophyletic. Adhesion factors with different mechanisms of colonization are associated with toxin and SR/AMR, indicating different ecotypes. LEE-effectors for adhesion presented in >70% of STEC (n=1409) but were rare in Enterotoxigenic *E. coli* (ETEC, n=70), presenting an opposite for F17 fimbriae. The antibiotic resistance, β-lactamase type TEM-1, was abundant in pathogenic *E. coli;* however, type OXA-1 enriched only in *Shigella/*Enteroinvasive *E. coli*. These distinctive genetic makeups infer the selective pressures that consistently shape bacterial strategies across different ecological niches. STEC is generally encoded for acid resistance but not for heat resistance, likely reflecting adaptation to the ruminant gut where acid stress but not heat is relevant as a selective pressure.

Significance: The findings deepen the understanding of the ecology of *E. coli*. Microbial control, including the implementation of antimicrobial intervention based on the stress resistance profiles of ecotypes, improves the mitigation of pathogenic *E. coli* in different environmental contexts.

T5-02 Comparative Analysis of Culture and Non-Culture Methods for the Detection of *Salmonella* in Pecan Orchard Soil

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❖ *Developing Scientist Entrant*

Introduction: *Salmonella enterica* is an important foodborne pathogen and its detection in food and environment has played a significant role in the management of food safety**.**

Purpose: This study aimed to compare the detection of *Salmonella* in pecan orchard soil samples by culture-dependent and culture-independent methods.

Methods: Twelve soil samples were collected from twelve different plots of pecan orchards. The culture method for detection of *Salmonella* involved enrichment, selective isolation, and multiplex PCR confirmation. For culture-independent detection, both high-throughput amplicon (16S rRNA gene) and shotgun sequencing were used. Total genomic DNA from the same soil samples were extracted using DNeasy PowerSoil Pro Kits. PCR amplification of bacteria-specific V4–V5 regions was performed, and positive amplicons were purified using AMpure XP beads and indexed with Nextera XT kit for 600 cycles of paired-end amplicon sequencing in Illumina MiSeq sequencer. Raw reads quality was checked using FastQC. Paired-end reads were merged and filtered using FLASH and Fastp, respectively. Raw tags were compared with the bacteria-specific BugBase. For shotgun sequencing, DNA library was prepared using the Nextera DNA Flex library preparation kit and sequencing was performed on an Illumina HiSeq platform. After quality check with FastQC, raw reads were assembled based on clean data using MEGAHIT.

Results: Culture-dependent method detected *Salmonella* in three among 12 samples investigated. For metagenomics approaches, the amplicon sequencing detected *Salmonella* sp. at a level of 0.0027±0.0029% in three soil samples (positive samples were different from those detected by culture method), whereas all soil samples were positive for *Salmonella enterica* by shotgun sequencing at a level of 0.0008±0.0008% per microbiome.

Significance: Our findings highlight the challenge in detection of foodborne pathogens in environmental samples by culture and non-culture methods.

T5-03 Development of an Electrochemical Genosensor for the *Dinophysis* spp.: Dinoflagellates Identification: A Breakthrough in Food Safety

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Introduction: The uncontrolled growth and proliferation of phytoplankton in aquatic systems can have detrimental effects on the aquatic environment by causing phytoplankton to produce harmful biotoxins, resulting in harmful algal blooms (HABs). This phenomenon adversely affects human and marine life and has a major impact on socio-economic sectors and resources that depend on clean water, such as tourism, industry, aquaculture and agriculture. HABs are mainly associated with a few groups of organisms, among which dinoflagellates stand out. These microorganisms can produce (bio)toxins which can have negative or lethal consequences on other species, such as fish, shellfish, and humans, through the absorption or ingestion of contaminated food or water.

Purpose*:* This study aimed to create a cost-effective platform to monitor *Dinophysis* dinoflagellates in environmental waters using electrochemical genosensors.

Methods*:* The device design involves three steps: (i) Formation of a mixed self-assembled monolayer on the electrode's surface, consisting of a linear DNA-capture probe and mercaptohexanol in the sensing phase; (ii) Promotion the hybridization of the complementary DNA sequence through a sandwich hybridization format assay; and (iii) Detection the DNA duplex using an enzymatic scheme for electrochemical signal amplification.

Results*:* Under optimal conditions, the genosensor exhibited a linear range of 0.1 to 1.0 nM, with a limit of detection (LOD) and limit of quantification (LOQ) of 12.945 and 43.148 pM, respectively, and reproducibility < 4%. Successful measurements of *Dinophysis* species in Atlantic Ocean waters, without purification after PCR amplification, underscore the genosensor's efficiency in detecting these dinoflagellates.

Significance: These findings suggest the potential for a low-cost device for monitoring harmful *Dinophysis* dinoflagellates and to anticipate and mitigate HABs.

T5-04 Genomic Characterization of a Reoccurring Strain of E. coli O157:H7 Associated with Multiple Sources Reveals a Highly Conserved Mutation within a Secreted Virulence Factor

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Introduction: Outbreaks or infections of the REPEXH01 strain of *Escherichia coli* O157:H7 have occurred every year since 2017 leading to its classification as a reoccurring, emerging, and persistent (REP) strain by the Centers for Disease Control and Prevention. Outbreaks have been associated with leafy greens, recreational water, and ground beef.

Purpose: To understand the emergence and defining molecular features of REPEHX01 we examined REPEXH01 genomes (n=181) and genomes from closely-related *E. coli* associated with human infections (*n* = 105) sequenced by the PulseNet USA Network.

Methods: A single nucleotide polymporphism (SNP) analysis was conducted using Lyve-SET. A time tree was generated in BEAST2. Antimicrobial resistance determinants (ARD) and plasmid replicons were identified using the ResFinder and PlasmidFinder databases, respectively. Isolates were further characterized through annotation with Prokka followed by analysis in Roary and Scoary to identify differences in the pangenome.

Results: Bioinformatic analysis of 286 isolates revealed several trends. REPEXH01 was estimated to have emerged late 2015 (95% HPD 3/5/2015 – 9/4/2016) just prior to the detection of clinical cases. ARD were widespread in these isolates: >99% of isolates were predicted to be resistant to aminoglycosides, sulfonamides, phenicols, tetracycline, and quaternary ammonium compounds (QACs). Most isolates harbored IncFIA and IncFIB replicons. A single base pair deletion in the secreted virulence effector protein *espW*, was strongly correlated ($P = 10^{-323}$) with REPEHX01. This deletion produces a frame shift that leads to early termination of the protein.

Significance: The REPEXH01 strain of *E. coli* O157:H7 associated with multiple sources emerged in late 2015 and has been the source of enteric illness since 2017. This strain possesses resistance to antimicrobials including QACs. A defining feature of this strain is a single base pair deletion in the virulence gene, *espW*. Additional work is needed to determine the significance of this mutation.

T5-05 Hollow Glass Microspheres Coated with Specific Antibodies Enable Rapid Isolation and Detection of *Salmonella* from Food

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❖ ***Developing Scientist Entrant***

Introduction: A rapid bacterial isolation and detection system is vital for reducing *Salmonella*-associated foodborne infections. Current bacterial culture-based methods are inaccessible in the resource-limited areas and are time-consuming. We propose use of self-floating hollow glass microspheres (HGMS) coated with anti-*Salmonella* antibodies conjugated layer-by-layer (LbL) nanostructured polymeric films for rapid isolation and recovery of *Salmonella.* This technique enables *Salmonella* isolation in 3h and confirmation within 24h, as opposed to 4-5 days required for the traditional methods.

Purpose: Development of a novel, inexpensive and rapid method for isolation and detection of *Salmonella* from food.

Methods: LbL self-assembly technique was used to coat HGMS (size=20μ, density=0.6g/cc) with alternate positive and negative charged polymeric layers, followed by biotin-neutravidin conjugation for antibody attachment. PBS, beef, cantaloupe, and milk samples spiked with *Salmonella typhimurium GFP* at various concentrations (50,000 – 500 CFU/mL) were mixed with coated-HGMS for 2h and uncaptured bacteria were removed by washing with PBS. Captured *Salmonella* were recovered by enzymatic degradation of the LbL film, plated on the agar plates for further growth and subjected to downstream MALDI analysis for isolate confirmation.

Results: The capture efficiency of *Salmonella* by the coated-HGMS was 18.7±3.4% with a detection limit of 500 CFU/mL. We observed a negligible non-specific binding of other bacteria ($p<0.05$) confirming its efficacy in selective isolation of *Salmonella* from food samples*.* The presence of captured *Salmonella* on the HGMS surface was confirmed by scanning electron microscopy (SEM). MALDI analysis confirmed the selective presence of *Salmonella*

surface proteins in isolates. Atomic force microscopy (AFM), Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) and profilometry confirmed successful deposition of LbL-film and bacterial capture and release.

Significance: HGMS coated with specific antibodies were shown to rapidly isolate and detect *Salmonella* from food. These encouraging preliminary results show the potential of this technique for detection of *Salmonella* from food in remote areas without any sophisticated equipment.

T5-06 A Fiber Optics SERS Sensor for Rapid Detection of *Salmonella* in Raw Poultry Products

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Introduction: *Salmonella* remains a leading cause of foodborne diseases in the U.S., causing millions of infections annually and costing the U.S. economy $4.1 billion. Relatively high cost, labor intensive and time-consuming traditional pathogen detection technologies have been major hurdles to efficient, rapid detection of *Salmonella*.

Purpose: To validate fiberoptics based Surface Enhanced Raman Spectroscopy (SERS) sensor to detect *Salmonella* in raw poultry rinsate samples

Methods: The sensor integrates highly uniform and repeatable nanoantenna arrays onto a side-polished multimode optical fiber core, significantly amplifying the detected light intensity. A 3D-printed microstructure with cylindrical ports at a 15° tilted angle maximizes reflected light, and a groove facilitates manual fiber core polishing. Housed in a 3D-printed chamber for sample loading, this system enables rapid, selective *Salmonella* detection by detecting Raman spectra fingerprint and comparing them with reference *Salmonella* spectra. This design presents a new solution, by increasing the sensing surface area, achieving much stronger signal-to-noise ratio compared to SERS sensors placed on fiber tip. A Low-cost Microsphere Photolithography was employed to pattern the nanoantenna arrays featuring multiple disks with diameters of 500-800 nm and a fixed periodicity of 1 µm.

Results: The sensor demonstrated high specificity, detecting *Salmonella* with a limit of detection of 10 cells/30ml in 10 minutes. It also detected 1 cell/ml. It achieved multiplex detection of *Salmonella* and *E. coli* O175:H7 and differentiated live from dead *Salmonella* cells treated with Peracetic Acid. The sensor with larger surface area (6 mm x 105 µm vs. 3 mm x 125 µm) achieved higher sensitivity.

Significance: This project aims to create a portable, cost-effective and user-friendly sensor for rapid *Salmonella* detection in poultry, enabling poultry companies to promptly implement *Salmonella* intervention to improve food safety. The SERS sensor can be adapted for other foodborne pathogens like beef, dairy, water products and clinical application.

T5-07 Leafy Green Microbiome Diversity on Urban Farms Reflects Site-Specific Conditions

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Introduction: Urban agriculture has become increasingly important to support food security. While bio-based amendments are widely used to restore soil health, especially with the goal of growing produce organically or pesticide free, impacts on the soil and plant microbiomes are not well established.

Purpose: To characterize the effects of crop management in combination with local environmental factors reflective of land history on microbiome diversity associated with leafy vegetable production on urban farms.

Methods: We conducted a molecular survey of leafy vegetable microbiomes (kale, lettuce, chard, cabbage) across urban agriculture systems in greater Washington, D.C. (n=7 sites). Microbial communities associated with the phyllosphere (n=92), rhizosphere (n=92), and bulk soil (n=39) were processed with16S rRNA gene sequencing and analyzed via QIIME2 and PICRUSt2. A variety of statistical approaches (e.g., PERMANOVA, mixed-effects models) were applied to explore differences in microbial community taxonomic and predicted functional diversity across and within sites as a factor of site-specific conditions.

Results: The rhizosphere and bulk soil microbiomes were dominated by Proteobacteria, Actinobacteria, and Acidobacteria, while the phyllosphere was dominated by the two former and Bacteroidetes. The rhizosphere was significantly enriched with Firmicutes, such as *Bacillus* ($P<0.001$), and less-abundant Chloroflexi ($p<0.001$) and Gemmatinomonadetes ($p<0.001$), suggesting important roles in plant-nutrient acquisition. Bacterial community diversity significantly differed across farms for all sample types ($p<0.05$). Taxonomic profiles and predicted functional potential of the microbiota further correlated with soil pH, amendment types, vegetable variety, plant growth stage, and irrigation resource (e.g., well, pond, city water), among other factors.

Significance: Crop management on urban farms, especially soil amendments, shapes the underlying bacterial communities. Future research will focus on microbial interactions with applications to enhance the quality and safety of produce in these emerging food systems.

T5-08 Long-Term Genomic Surveillance Shows Agricultural Surface Waters are an Increasingly Important Reservoir of Multi-Drug-Resistant, Non-Typhoidal *Salmonella* in Central Mexico

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Introduction: Agricultural surface waters (ASW) may introduce multi-drug resistant (MDR) *Salmonella enterica* into the food chain, posing a risk to food safety and public health.

Purpose: To characterize the role of ASW from Central Mexico as a reservoir of SE exhibiting antimicrobial resistance profiles of human clinical significance.

Methods: We conducted antibiotic susceptibility testing (disk diffusion method) and comparative genomic analysis of 516 *S. enterica* isolates of 55 different serovars collected from 227 ASW samples across 49 watersheds of Central Mexico from June 2019 to May 2022. These isolates were sequenced using the Illumina MiSeq or NextSeq platforms. Assembled genomes were used to predict AMR genotypes using AMRFinderPlus 3.10 software. We tested the association between MDR phenotypes, serovars, and the period of collection through the chi-square test and calculated the Pearson correlation coefficient between phenotypic and genotypic resistance.

Results*:* Phenotypic resistance was more frequently observed for streptomycin (31%), tetracycline (30%), chloramphenicol (22%), trimethoprim-sulfamethoxazole (21%), and ampicillin (20%). Nearly 30% of the isolates had MDR phenotypes, which were observed in higher proportions in the post COVID-19 pandemic period (2021-2022): $\chi^2=13.5$, P=0.0002. AMR phenotypes and genotypes correlated well (r=0.95), and MDR isolates carried multiple resistance alleles affecting aminoglycosides (*aadA, aac, aph*), betalactams (*blaCARB, blaTEM, blaCTX-M, blaCMY*), phenicols (*floR, cmlA*), folate pathway inhibitors (*sul, dfrA*), tetracyclines (*tetABCDM*), macrolides [*mph(A)*], and quinolones (*qnrABS*, *oqxAB*, and point mutations in *gyrA*: S83F, S83Y, D87Y, and *parE*: H462Y), with an overall AMR gene diversity of 22. MDR profiles were more frequent in isolates of epidemiologically relevant serovars such as Typhimurium, 1,4,[5],12:i:-, Newport, and Senftenberg ($\chi^2=11.6$, P=0.0007).

Significance: ASW from Central Mexico are a relevant source of MDR *Salmonella* and a wide diversity of AMR genes of clinical significance. Monitoring and treating ASW before use are essential to mitigate contamination of produce and human exposure to MDR pathogens.

T5-09 Novel CRISPR-RNase-Based Method For Detection of Potentially Infectious Viruses in Produce

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❖ ***Developing Scientist Entrant***

Introduction: Standard RT-qPCR methods detect genomic material of foodborne viruses but do not distinguish infectious and non-infectious forms.

Purpose: To detect a potentially infectious human norovirus surrogate, murine norovirus (MNV-1), in fresh produce, we developed an integrated CRISPR-Cas13a-RNase assay.

Methods: Lettuce and blueberries samples were spiked with a serial MNV-1 dilution (5.06×10^5 to 5.06 gc/rx). The ISO 15216-1:2017 method for viral elution-concentration and RT-qPCR was followed. Prior to RNA extraction, free viral RNA was degraded with RNase ONE™ (Promega). Cas13a detection consisted of isothermal RPA pre-amplification (TwistDx™), T7-transcription and detection by gRNA in a one-step reaction. Recovery efficiencies were evaluated in RT-qPCR and CRISPR RNase pre-treated and non-pretreated samples where a t-test for independent samples was applied.

Results: In non-RNase-treated spiked lettuce, RT-qPCR and CRISPR assays detected all viral spiked dilutions, however, in RNase treated samples, the CRISPR assay detected the 5.06×10^1 gc/rx dilution while the RT-qPCR assay only detected the 5.06×10^2 gc/rx dilution. In non-RNase treated spiked lettuce, the CRISPR assay, compared to RT-qPCR, exhibited ten-fold higher recovery yields and, in RNase-pretreated samples, more than fifty-fold higher recovery yields ($p<0.0001$). In non-RNase treated spiked blueberries, the CRISPR and RT-qPCR assays both detected the 5.06×10^1 gc/rx dilution. In RNase pre-treated samples, the CRISPR assay detected the 5.06×10^1 gc/rx dilution outperforming the RT-qPCR assay, which only detected the 5.06×10^4 gc/rx dilution. In blueberries, the CRISPR, compared to RT-qPCR, assay, showed a higher recovery percentage in non-RNase treated (80 times) and RNase-treated (40 times) samples ($p<0.0001$).

Significance: This novel assay provides a pathway for the detection of infectious foodborne viruses, such as human norovirus and hepatitis A virus, strengthening the agricultural produce safety system by enabling decision-making based on real infection risk.

T5-10 Species Identification and Strain Discrimination of Fermentation Yeasts Using Raman Spectroscopy Combined with Convolutional Neural Networks

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❖ ***Developing Scientist Entrant***

Introduction: *Saccharomyces cerevisiae* and *Saccharomyces uvarum* are the commonly used yeast species in wine production and cider fermentation. Reliable typing of these yeast strains is crucial for fermentation industry to monitor the authenticity of yeast starters and ensure a reliable fermentation process and high-quality final products.

Purpose*:* This study aimed to develop a rapid and reliable approach of using Raman spectroscopy and convolutional neural networks (CNNs) to achieve accurate identification of *S. cerevisiae* and *S. uvarum* at both species and strain levels.

Methods*:* A total of 12 *S. cerevisiae* and 15 *S. uvarum* strains isolated from various sources were included and Raman spectra were collected from each strain. Random forest and CNN models were utilized to discriminate yeast isolates at the species level and strain level, respectively. Eight yeast strains were spiked in grape juice and identified using Raman spectroscopy to investigate its feasibility for wine fermentation. The quantification capability of Raman-CNN was further assessed by detecting *S. cerevisiae* EC1118 in a yeast cocktail with different concentrations.

Results*: S. cerevisiae* and *S. uvarum* strains were classified at the species level with a high accuracy of 98.9% using random forest. Cultivation time and temperature did not significantly affect the spectral reproducibility and discrimination capability. An overall accuracy of 91.9% was achieved to discriminate 27 yeast isolates at the strain level using a CNN model. Raman-CNN further identified 8 yeast isolates spiked in grape juice with an accuracy of 98.1%. This approach also precisely quantified a specific yeast strain within a yeast mixture with a R^2 of 0.9913 and an average error of 4.09%.

Significance: Raman spectroscopy combined with CNN represents a rapid, non-destructive and reliable strain typing for *S. cerevisiae* and *S. uvarum*. This approach can be utilized to test the identity of commercialized dry yeast products and monitor the diversity of yeast strains during fermentation, providing a high-throughput screening method for agri-food industry.

T5-11 The Phylogeny of the Top 100 Most Prevalent *Salmonella* Serovars on NCBI Pathogen Detection

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❖ ***Developing Scientist Entrant***

Introduction: Although serotyping is a major subtyping method for *Salmonella*, previous studies have shown that isolates of the same serovar may have evolved independently multiple times and present distinct epidemiological characteristics.

Purpose: The goal of this project was to identify which serovars are not monophyletic and to identify poly- or paraphyletic lineages within the top 100 most prevalent *Salmonella* serovars on the NCBI Pathogen Detection database using single nucleotide polymorphism (SNP) differences.

Methods*:* For each serovar, we identified all singletons and selected one representative isolate from each NCBI Pathogen Detection SNP cluster. These selected isolates were then subjected to *in silico* serotyping using a custom pipeline involving SISTR and SeqSero2 to confirm their serotypes. Subsequently, kSNP4 and FastTree were used to reconstruct a maximum likelihood phylogeny based on the core SNP matrix of confirmed isolates. To facilitate exploration of the results, a web-based, interactive tree with a search function was created using the Shiny package in R.

Results: Among the top 20 most prevalent serovars on NCBI Pathogen Detection, *Salmonella* Javiana, Typhi, Heidelberg, Anatum, and Dublin are the only monophyletic serovars, while the remaining 15 are polyphyletic. Each of these 15 polyphyletic serovars were found to have between 2 and 7 phylogenetic groups (i.e., clades), with a median number of 4 clades per serovar. Interestingly, 3 of the clades of the monophasic Typhimurium variant (I 4,5,12:i:-), do not cluster with a serovar Typhimurium (I 4,5,12:i:1,2) clades, suggesting that not all monophasic Typhimurium have emerged from biphasic Typhimurium.

Significance: These findings will facilitate a better understanding not only of serovar conversion, but also of evolutionary patterns of serovars commonly isolated from clinical cases and environments. Our results suggest that current risk assessment of *Salmonella* should shift from a serovar-based classification to phylogenetic-based approaches.

T5-12 Understanding the Microbiome and Exploring Early Detection of Pathogens in Microgreens Grown from Seeds Contaminated with *Escherichia coli* O157:H7, *Salmonella enterica, and Listeria monocytogenes* Serovars Using Shotgun Metagenomics

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Introduction: Native microbiota can influence the survival of foodborne pathogens both internally and on the surface of leafy greens. Microgreens are novel salad greens that have gained popularity. Being grown in controlled conditions, these greens can harbor various microbial communities as well as sustain the growth of several pathogens.

Purpose: The study aims to understand the microbial communities that are prevalent in microgreens which can lead to understanding how foodborne

pathogens can survive in these commonly consumed commodities as well as explore early detection of pathogens in these microgreens using shotgun metagenomics.

Methods: Seeds of daikon, mustard, broccoli, and red cabbage microgreens were inoculated with a cocktail of two strains of *Salmonella*, *E. coli* O157:H7 or three strains of *Listeria monocytogenes* at low and high inoculum levels and allowed to germinate on soil beds. On days 0, 7 and 14, microgreen/seed and soil samples were harvested, enriched in TSB and DNA was extracted using Promega RSC cultured cell kit. Shotgun metagenomic libraries were prepared using Illumina DNA prep library kit and sequenced using Illumina Nextseq2000. The metagenomic reads were analyzed using kraken2, bettercallsal analysis pipeline and custom kmer analysis developed in-house within FDA.

Results: A distinct difference was observed in the microbial diversity of the four microgreens and soil samples; *Pseudomonas* was predominant in all samples. The relative abundance maps indicated the presence of other genera such as *Bacillus, Clostridium, Pantoea,* and *Sporosarcina* among others. The target pathogen pipelines identified the presence of the exact serovars used in the study in microgreen as well as soil samples.

Significance: Understanding the role of microbiome and the early detection of pathogens using quasi metagenomic approach from complex food and environmental matrices seems essential for successful outbreak investigation pertaining to public health in the genomics era.

T6-01 Modeling the Effect of Pre-Inoculation Temperature History on Growth Kinetics of *Listeria monocytogenes*

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Introduction: Adaptation of the bacteria to different environments is often determined by the pre-inoculation conditions, influencing the duration of lag phase. Even though it is a phase of zero growth, its duration is crucial for determining the safety of foods. Temperature history has often been the primary component of pre-inoculation conditions, affecting the lag time of bacteria upon transfer to the new environment.

Purpose: To assess the impact of pre-inoculation temperature history on the lag phase duration (LPD) of five strains of *Listeria monocytogenes* in TSB.

Methods: Five strains of *Listeria monocytogenes* were used; three were obtained from the cantaloupe outbreak, and the other two were from the raw diced yellow onion and celery outbreak. The effect of the pre and post-inoculation isothermal temperatures (8, 15, 20, 30, 40℃) was measured as the optical density measurements using Bio-Tek Cytation3 image reader. During the pre-inoculation stage, 96-well plates were incubated at all five pre-inoculation temperatures, followed by the transfer of exponentially growing cells at each pre-inoculation temperature to all five post-inoculation temperatures. The LPD was measured with the Baranyi & Roberts primary model.

Results: Overall, out of all the five strains, LPD was higher in four strains at low post-inoculation temperatures of 8 (116-151 hr.) and 15℃ (50-65 hr.) as compared to 20, 30, 40℃ ($p<0.05$). However, varied results were observed for the effect of pre-inoculation temperature in each strain. Due to pre-inoculation at 8, 15, 20℃, LPD was significantly reduced when post-incubated at low temperatures, 8 and 15℃ ($p<0.05$). Temperature history did not significantly affect lag time at post-inoculation temperatures of 20, 30, 40℃ ($p>0.05$).

Significance: Predictive models can accurately predict the growth rate of pathogens, but poor estimates are obtained for LPD. Therefore, this study will help determine the effect of pre-inoculation temperature history for accurate predictions of LPD.

T6-02 A Comparison of Machine Learning Methods in Predicting *Salmonella* Foodborne Pathogen Positive Rates in FSIS-Inspected Raw Pork Products and Variable Association with Establishment Process Control Measures

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Introduction: Pork is a major source of foodborne salmonellosis. The Interagency Food Safety and Analytics Collaboration estimated that 12.8% of foodborne salmonellosis was attributed to pork in 2020. The Food Safety and Inspection Service (FSIS) conducted a pork Baseline Study from June 2017-May 2019. Data included total *Salmonella* isolates, positive serotypes, and antimicrobial resistance (AMR) for establishment characteristics and process control measures for comminuted, intact, and non-intact pork products.

Purpose*:* Prediction of *Salmonella* positive rates in each pork product using the best predictive machine learning (ML) model, additionally linking important ML model variables to *Salmonella* serotypes and AMR characteristics associated with establishment process control measures.

Methods*:* The ML models for stochastic gradient boosting, Logistic Regression, k-Nearest Neighbor, and random forest (RF) exclusively applying synthetic minority over-sampling (SMOTE) were compared. Most important variables were identified and associated with *Salmonella* serotypes with AMR characteristics.

Results*: Salmonella* positive isolates were identified in 287 total establishments (n=8,014, 13.8% positive) for all pork products, 147 establishments for comminuted (n=3288, 21.5% positive), 139 establishments for intact (n=2375, 9.2% positive), and in 54 establishments for non-intact (n=2351, 7.8% positive). The variables for production volume, generic *E. coli,* aerobic plate count, hot-boned, fresh comminuted, and ground products were most important and the serotypes with AMR (rank) for Anatum (3), Infantis (6), Derby (2), Johannesburg (5), I 4,[5],12:i:- (1), and Typhimurium (4) were most frequent across all datasets. The SMOTE RF model provided the best important variable ranking method in all datasets.

Significance: This data-driven approach for picking variables for analysis, rather than selecting variables based on prior knowledge, allows for selection of study variables without any preconceived notion of the data. Further multivariable factor analysis and logistic regression modeling of *Salmonella* data could help to identify additional factors that contribute to *Salmonella* in raw pork products.

T6-03 Comprehensive Analysis of *Listeria monocytogenes* Growth in Ready-to-Eat Fish and Meat Products: Understanding Variability to Assess Public Health Risks

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Introduction: *Listeria monocytogenes* remains a significant global foodborne issue, severely impacting public health, although improvements to prevent this foodborne hazard are currently ongoing. Outbreaks are frequently associated with Ready-to-Eat (RTE) products, particularly those with a relatively extended shelf life, mostly RTE fish and meat products.

Purpose: The development of a comprehensive database for the assessment of *L. monocytogenes* growth variability and influencing factors in different categories of RTE fish and meat products.

Methods: An extensive literature search was conducted through ComBase browser and the main scientific bibliographical databases (Web of Science, PubMed, Scopus). RTE fish selected in the search included hot-smoked, cold-smoked, salted products, and fish pâté, while RTE meat products comprised cooked ham, cooked meat, cooked sausages, and meat pâté. The records were examined, with maximum growth rates (μ_{max}) either extracted directly or calculated from microbial counts, and standardized at 5 °C. Type of product, food characteristics, and source were recorded. Results were incorporated into a growth values dataset previously generated among a project awarded by EFSA. Finally, a descriptive analysis was carried out to summarize the collected data.

Results: A total of 25 sources covered RTE fish, providing 210 growth values, primarily centered around cold-smoked products (μmax at 5°C: 0.000-0.124 log CFU/h). Hot-smoked (0.004-0.026 log CFU/h), salted products (0.001-0.021 log CFU/h), and fish pâté (0.025-0.088 log CFU/h) constituted the remaining third. For RTE meat, 78 sources contributed 1358 growth values, with 37.8% attributed to cooked ham (0.001-0.386 log CFU/h), 26.1% to cooked RTE meat (0.00002-0.136 log CFU/h), 24.7% to RTE cooked sausages (0.001-0.182 log CFU/h), and 11.4% to meat pâté (0.009-0.513 log CFU/h).

Significance: By compiling growth rate data across various RTE product types, the study facilitates comprehensive risk assessments and management

strategies for *L. monocytogenes*. This informs stakeholders in implementing preventive measures to minimize *Listeria* contamination risks and safeguard public health.

T6-04 Continuous Improvement of the Canadian Food Inspection Agency's Data-Driven Food Safety Risk Assessment Tool for Hatcheries: A New Approach for the Source Attribution of *Salmonella* Illnesses

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Introduction: The Canadian Food Inspection Agency has developed a risk assessment tool (ERA-Hatchery model) to evaluate hatcheries based on their food safety risk related to *Salmonella* spp. and to allocate inspection resources accordingly.

Purpose: The objective was to refine the relative risk (RR) of each bird types to the food safety burden by considering the capacities of federally regulated hatcheries.

Methodology: An expert elicitation was performed using an interactive Excel questionnaire. Experts were asked to allocate the *Salmonella* spp. burden in the poultry meat and table-egg production chains at the bird type level considering the national poultry production volume as a measure of exposure. This innovative approach allowed comparing the contribution of each bird type to the *Salmonella* spp. burden on an equal production basis. Real-time calculations of the RR attributed by experts enabled live adjustments to suitably express the risk attribution. Weighted medians and 95% confidence intervals were calculated.

Results: The questionnaire was completed by eight experts from May to August 2022. Compared to the 2017 study (Racicot and al., 2020), new source attribution estimates resulted in a reduction of the burden associated to broiler breeders (2.4x) and layer breeders (4.2x), both showing the lowest Canadian hatchery production volume for the poultry meat and table-egg production chains, respectively. Results also showed an increase in the RR associated with turkey (1.3x) for the poultry meat production chain. Broiler breeders remain the type of birds representing the highest RR for the poultry meat chain, whereas layers have replaced layer breeders as the birds with the highest RR within the table-egg chain.

Significance: Results have been incorporated into an automated IT solution designed for the ERA-Hatchery model able to assist the Agency in decision-making and thus improving the risk-informed allocation of inspection resources based on the food safety burden attributed to each hatchery in a timely manner.

T6-05 Overview of the Food Safety and Inspection Service's *Salmonella* Framework Initiative and Risk Assessments

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Introduction: Despite the Food Safety and Inspection Service (FSIS) sampling data showing reductions in *Salmonella* contamination in poultry products, salmonellosis infections associated with poultry consumption have not decreased. In November 2022, FSIS announced a proposed regulatory framework to control *Salmonella* in poultry products to reduce foodborne *Salmonella* infections linked to chicken and turkey consumption. To support this approach, FSIS conducted quantitative probabilistic microbial risk assessments for *Salmonella* in chicken and turkey. These risk assessments provide necessary scientific support for rule making and are required by the United States federal rule making processes.

Purpose: This presentation will provide an overview of risk management questions, and the three major components—enforceable final product standards, process control, and receiving—of the two risk assessments conducted.

Methods: The relationship between the amount of *Salmonella* and the presence of certain *Salmonella* serotypes on chicken received for slaughter and/or on chicken products and the probability of foodborne illness was explored, as well as the relationship between changes in microbiological indicator organisms on chicken carcasses from rehang to post-chill and changes in foodborne illnesses. Bioinformatics were used to cluster *Salmonella* serotypes according to virulence-associated gene markers.

Results: Results will be shared when the proposed Salmonella Framework Rule is published, per Office of Management and Budget rules.

Significance: FSIS' new framework to controlling *Salmonella* will help reduce the number of *Salmonella* infections linked to poultry consumption. This initiative marks a major advancement in FSIS' pathogen reduction strategy for *Salmonella*.

T6-06 Identifying *Salmonella* Serotypes of Concern to Target for Control to Reduce the Risk of Salmonellosis

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Introduction: There is an increasing awareness in the field of *Salmonella* epidemiology that focusing control efforts on those serotypes which cause severe human health outcomes, as opposed to broadly targeting all *Salmonella,* will lead to the advances in decreasing the incidence of salmonellosis, yet little guidance exists to support validated selection of target serotypes.

Purpose: We develop an approach to identifying serotypes of greater concern and present a case study using meat- and poultry-attributed outbreaks to examine challenges in developing a standardized framework for defining target serotypes.

Methods: This is an analysis of pre-existing epidemiology data. Data were downloaded from the Center for Disease Control and Prevention's National Outbreak Reporting System describing 227 meat-attributed outbreaks of salmonellosis from 2009 to 2021. Variables analyzed included the serotype, meat source, number of illnesses, and number of hospitalizations. We used this information to identify the most hazardous *Salmonella* serotypes for human health, using a traditional outlier approach (interquartile range) and a machine learning approach (agglomerative nested cluster analysis).

Results: Serotypes of concern were identified for beef, chicken, pork, turkey, and for these four commodities combined. The machine learning and outlier approaches had overlap but also identified different serotypes of concern. In total, 21 serotypes were identified as "of concern", representing 95% of all illnesses in the dataset. We discuss the challenges associated with these analyses to provide guidelines for researchers and industry.

Significance: We provide guidelines based on the analysis of epidemiological data for targeting specific *Salmonella* for management in the meat industry, as opposed to broadly targeting all *Salmonella*. Development of new detection and management tools which target a suite of *Salmonella* could use this list for validation to ensure the tool will have the necessary public health impacts when implemented.

T6-07 Leveraging *Salmonella S*urveillance Data to Optimize Plant Level Controls: A Case for Moving from Hazards to Risks

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Introduction: Current *Salmonella* surveillance programs in beef processing are based on pathogen prevalence, which does not fully address the potential health and legal risks from pathogens in the plant.
Purpose: demonstrate the use of *Salmonella* data to estimate individual plant food safety performance against a national benchmark and evaluate efficacy of future plant-level sampling and interventions.
Methods: Publicly available USDA-FSIS *Salmonella* surveillance data from 2016-2019 in ground beef facilities was used to model national benchmark indicators of the risk of salmonellosis from beef. Metrics from six hypothetical large-volume ground beef plants with diverse *Salmonella* profiles (a "high-risk" plant with elevated prevalence of higher virulence (HV) and average lower virulence (LV), one with high LV and average HV, and four others with similar prevalences to national levels) were compared against the benchmarks of illnesses per product volume and annual illnesses attributed per facility. The costs were estimated and compared between status quo and interventions, assuming an annual hypothetical budget of $750k. Interventions combined increased test-and-diversions or log reductions of microbial load.
Results: The highest-risk plant with elevated HV prevalence (2.5% (95% CI 1.3-3.9)) compared with national prevalence (0.64% (0.49-0.82) resulted in 2.5 (1.0-4.0) more volume-adjusted illnesses from any *Salmonella* than the national benchmark. Elevated LV prevalence (5.2% (3.8-6.6)) relative to national (1.6% (1.3-1.9)) did not produce excess illnesses (ratio of 1.2 (0.4-2.0)). Decreasing *Salmonella* load by two logs, or increasing testing to at least 75% in the summer season were most cost-effective (Extra $175/illness prevented and extra $316/illness, respectively) and decreased predicted illnesses from the highest-risk plant to be comparable (RR 0.82 (0.74-0.89) and 1.75 (0.41-3.2)) to the national average ($1.3x10^{-6}$ ($4.3x10^{-7}$- $4.1x10^{-6}$) illnesses/lb) while within budget.
Significance: This analysis demonstrates a patent-pending method for processor decision-making around pathogen surveillance and control that can result in cost-effective solutions for foodborne illnesses.

T6-08 Machine Learning to Identify and Predict *Salmonella* Genetic Patterns Associated with Stages of Chicken Production and Processing

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Introduction: *Salmonella enterica* is a foodborne pathogen with major public health impact. A large number of *Salmonella* serovars have been implicated in human infections, with chicken remaining a significant foodborne source of infection. However, models predicting *Salmonella* behavior in the food production and processing ecosystems do not consider genome-level differences in these serovars.
Purpose: The aim of this study was to determine the applicability of machine learning in delineating genome-level differences in *Salmonella* isolated from different stages of chicken production and processing.
Methods: Genome assemblies of *Salmonella* isolates obtained from different stages of chicken production and processing were obtained from the National Center for Biotechnology Information's (NCBI) Pathogen Detection database. The sequences were assembled and annotated on Bacterial and Viral Bioinformatics Resource Center and the *Salmonella* pangenome assembled on Python. Machine learning algorithms, including LogitBoost, random forest (RF), and support vector machine, were used to predict *Salmonella* genetic patterns associated with different stages of chicken production and processing. The best-fit model was identified by analyzing the area under the receiver operating characteristic curve (AUROC) and accuracy scores.
Results: The identified *Salmonella* isolates (n=31,054) yielded a pangenome with >25,000 genes. All of the tested models showed acceptable classification accuracies (>0.70), and the best performing model (RF) demonstrated accuracy and AUROC scores of 0.72 and 0.82, respectively. Genes from the variable genome associated with virulence, stress response, metabolism, and cellular processes were identified as being important predictors of the stage of chicken production and processing.
Significance: This study is an initial attempt to employ machine learning to predict *Salmonella* genetic patterns associated with chicken production and processing. Foodborne salmonellosis cases have been historically associated with chicken and chicken products. Therefore, predicting *Salmonella* behavior across the chicken production and processing stages would have potential applications in mitigating the risk of *Salmonella* in poultry.

T6-09 Systematic Review and Meta-Analysis of the Impact of High-Pressure Processing on Microbial Inactivation in Raw Chicken

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❖ ***Developing Scientist Entrant***

Introduction: Following the USDA's declaration of *Salmonella* as an adulterant in the raw chicken component of breaded stuffed chicken products, there is an urgent need for the poultry industry to enhance their microbial intervention strategies. High-pressure processing (HPP) is a nonthermal process used to advance microbial safety and extend the shelf life of foods. However, research into pathogen inactivation in raw chicken by HPP, particularly using aggregated data, remains limited.
Purpose: Through systematic review and meta-analysis to evaluate the effect of HPP on the inactivation of common pathogenic and indicator bacteria in raw chicken, including *Salmonella* spp., generic *Escherichia coli*, and *Listeria monocytogenes*.
Methods: Web of Science and Scopus were searched using various terms related to chicken and HPP. After a two-phase screening (title/abstract and full-text), data from relevant studies were used to perform random-effects meta-analyses to estimate log reductions influenced by pressure, dwell time, and temperature. Additionally, a mixed-effect model was applied to quantify the relationship between log reductions and influential factors.
Results: The search identified 599 records, of which 18 were selected for meta-analyses. These studies evaluated various pressure levels (100-600 MPa), time (0.02-25 min), and temperature (4-40 °C). Meta-analyses showed that microbial log reductions increase with pressure, with mean reductions of 1.40, 3.57, and 6.11 for pressures <400 MPa, 400-600 MPa, and ≥600 MPa, respectively. Increased time positively correlated with greater reductions, while temperature seems to have no significant effect. Most studies focused on *Salmonella* and *E. coli*, showing similar reduction levels at pressure levels between 400 and 600 MPa but significantly greater reductions in *Salmonella* than *E. coli* at lower pressures (<400 MPa).
Significance: These findings provide the scientific community, poultry industry, and food safety authorities with evidence-based insights to optimize the effectiveness of HPP for microbial inactivation in raw chicken.

T6-10 FSIS *Salmonella* in Poultry Risk Assessments: Enforceable Final Product Standards

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Introduction: In October 2022, the Food Safety and Inspection Service (FSIS) announced a proposed regulatory framework to control *Salmonella* in poultry products to reduce foodborne *Salmonella* infections linked to chicken and turkey consumption. To support this new approach, FSIS conducted quantitative probabilistic microbial risk assessments for *Salmonella* in chicken and turkey.
Purpose: This presentation will provide an overview of, and results from, the risk management question addressing enforceable final product standards for chicken and turkey products.
Methods: The relationship between the amount of *Salmonella* and the presence of certain *Salmonella* serotypes on poultry received for slaughter and/or on chicken products (carcasses, parts, and comminuted) and turkey products (comminuted) and the probability of foodborne illness was estimated. Bioinformatics were used to cluster *Salmonella* serotypes according to virulence-associated gene markers. The major model inputs were systematically analyzed and used to develop a robust uncertainty analysis.
Results: Results will be shared when the proposed Salmonella Framework Rule is published, per Office of Management and Budget rules.
Significance: The risk assessments, along with additional scientific evidence produced by FSIS, provide the scientific justification for the proposed Salmonella framework rule for chicken and turkey.

T6-11 Prediction and Selection of Genetic Biomarkers of Microbial Stress Response Using Whole Genome Sequencing Data and Network-Diffusion Approach

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Introduction: Despite gains in accurate subtyping of microbial hazards using high-throughput DNA sequencing technologies (WGS), the incorporation of this data in predictive microbiology remains scantly explored as the high dimensionality and structural complexity of WGS data makes statistical analysis of WGS data non-trivial.
Purpose: This study explored network-diffusion based analysis (ND) with a case of *Listeria monocytogenes* using WGS for reproducible and biologically meaningful associations between gene variability and phenotypic properties considering the response to the four stress conditions: desiccation, salt, pH and low temperature.
Methods: The pangenome (7343 genes) from *L. monocytogenes* isolates (n=165) were converted to amino acid sequences and used in the ND analysis to identify genetic network regions associated with biological functions and stress response phenotype classes, i.e., highly susceptible, susceptible, tolerant and highly tolerant. Protein-protein networks were constructed for the individual stress response phenotype classes and an aggregated analysis combining all the four stress conditions.
Results: There was an aggregated module of 193 genes 80 of which (41.5%) were stress-phenotype specific. In particular, 7 (3.6%), 7 (3.6%), 36 (18.6%), and 30 (15.5%) genes were associated with a shift towards acid, cold, salt and desiccation stress tolerance, respectively. ND enabled to successfully detect significantly enriched protein network pathways previously associated with stress tolerance. The key functions of groups of proteins mediating stress tolerance against single or multiple stress conditions using ND indicated their possible role in metabolic pathways, biosynthesis, terpenoid backbone, and ubiquinone and other terpenoid-quinone, phosphotransferase system, ABC transporters and nucleotide excision repair.
Significance: The predictive modeling approach allowed to capture variability in microbial growth at strain or subgroup level. ND approach provides an opportunity for timely recognition of strains harboring novel virulence and environmental stress tolerance genetic elements which may be instrumental in supporting early outbreak detection and resolution.

T6-12 The Signal Assessment Process within the Canadian Food Inspection Agency

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Introduction: With a dynamic, global food risk landscape, competent authorities need to find a way to identify emerging issues to support food program risk management decisions.
Purpose: The Canadian Food Inspection Agency developed a signal assessment process to: 1) Enhance situational awareness of the global food landscape; 2) Build foresight capacity; and 3) Support a proactive risk management approach.
Methods: This process maximizes the use of available internal and external information to identify potential signals, which must meet established criteria. Signals are reviewed systematically by subject matter experts, prior to being communicated to an internal decision body for prioritization, further assessment, and recommendation for program management consideration. The signal, supporting documentation and corresponding program impact is recorded in a repository, which is used as an evergreen knowledge center.
Results: This process has established consistency in how signals are generated and used to inform food program management decisions, and since its launch in 2022, over 30 signals have been identified. This has positively impacted the food program through supporting program decisions, including: 1) Triggering the review of control measures to manage specific food safety risks, such as controls for imported infant formula; 2) Consideration of potential new policies on precautionary labelling, such as for undeclared milk allergens in dark chocolate; and 3) Identifying future areas of intelligence focus, such as products derived from cellular agriculture.
Significance: This work has strengthened: 1) Program agility and early awareness of potential issues; 2) Confidence that the potential impacts of a signal have been assessed and acted on appropriately; 3) Multi-faceted analysis of risks to public health and consumer protection, as well as risks to trade, reputation and public confidence related to certain emerging issues or changes in the known risk landscape; and 4) Capacity to proactively identify future potential vulnerabilities.

T7-01 *Salmonella*'s Transfer Potential Between Intact and Damaged Tomatoes and New and Used Harvest Bin Materials during Harvesting

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❖ ***Developing Scientist Entrant***

Introduction: Tomatoes have been associated with *Salmonella* outbreaks in the U.S.
Purpose: The purpose of this study was to evaluate *Salmonella*'s transfer potential between intact and damaged tomatoes and new and used harvest bin materials.
Methods: Intact and damaged tomatoes, and new and used HDPE, wood, or cardboard coupons were spot inoculated with a Rifampicin resistant

Salmonella cocktail (10^5) and dried for 1h. Uninoculated and inoculated items were placed into contact, compressed with a 1lb weight, for 10min, 3, 6, and 24h. *Salmonella* populations on both items were enumerated following a shake, rub, shake (30s each), dilutions, plating onto non-selective media with Rifampicin, and incubation (35°C, 24h). The experiment was replicated 3 times with 3 samples (n=9). The CFU/mL transferred to the uninoculated surface was divided by the CFU/mL on the inoculated surface and reported as log% TCs.

Results: *Salmonella* transfer to (range 0.897-2.005 log%) and from (0.250-2.044 log%) damaged tomatoes and used bin materials were significantly ($P \leq 0.05$) greater than the transfers to (-1.111-0.000 log%) and from (-0.847-0.000 log%) intact tomatoes and used bin materials, across all time points. There were no significant differences in transfer between intact and damage tomatoes, to (1.946-2.092 log%) and from (823-2.096 log%) new HDPE, across all contact times. There were significant ($p \leq 0.05$) transfer differences between intact and damaged tomatoes to new: wood at 3 (1.387 and 2.158 log%), 6 (0.000 and 2.107 log%), and 24h (0.000 and 2.069 log%), and to cardboard at 6h (0.000 and 2.259 log%). There were significant ($p \leq 0.05$) transfer differences between intact and damaged tomatoes from new wood at 6h (0.000 and 1.984 log%), and cardboard at 10min (1.930 and 0.000 log%), 3 (1.216 and 0.000 log%), and 6h (0.000 and 2.383 log%).

Significance: Transfer potential increases with damaged tomatoes and used surfaces; damaged tomatoes should not be harvested or picked up, and harvest bins should be maintained.

T7-02 Accelerating Pathogen Die-Off on Leafy Greens through LED Grow Light Spectral Modulation in Controlled Environment Agriculture

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Introduction: Controlled environment agriculture (CEA) has unique food safety improvement opportunities and risk profiles. The effects of LED grow lights on photosynthesis and crop yield have been extensively studied. However, little is known regarding the impacts of light quality (spectra profile) and quantity (light intensity and photoperiod) on pathogen survival, growth, or die-off on leafy greens.

Purpose: To evaluate the effects of different light treatments on the survival, growth, or die-off of *Listeria innocua* on baby kale plant during growth and during chlorine wash post-harvest. Each treatment consists 6 replications with 3-5 plants per replications.

Methods: Baby kale was grown in a deep-water culture system under a white spectrum (200 µmol/m^2/s PPFD) and 16 h photoperiod until Day 25. *L. innocua* was inoculated onto the adaxial surface of the leaves, after which the plants were exposed to blue, red, and white light treatments. Leaves were harvested 1 and 3 d later and subjected to wash (100 ppm chlorine, pH 6.5) and no wash treatments, followed by *L. innocua* enumeration. Aerobic bacterial counts (APC) were also determined using uninoculated leaves. Leaf weight, color, and morphology were also determined.

Results: Inoculated leaves exposed to blue light showed significantly ($p<0.05$) lower count of *L. innocua* than those treated with red or white light. The same trend was observed at both exposure intervals (1 or 3 d) and with or without washing. The APC level did not differ significantly among light treatment. Kale leaves exposed to different lights showed no difference in fresh weight, although red light produced leaves with lighter and yellower color.

Significance: Findings reveal, for the first time, the potential of using LED blue grow light to improve food safety of leafy green by accelerating pathogen die-off during plant growth in CEA.

T7-03 Adaptation of Shiga Toxin-Producing *Escherichia coli* to Fresh Produce Environment; Sprouts as an Example

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❖ ***Developing Scientist Entrant***

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) is a common cause of foodborne illnesses associated with ingestion of fresh produce, especially sprouted seeds.

Purpose: Monitoring the colonization and virulence behavior of STEC during alfalfa sprouting and refrigerated storage of sprouts, and whether STEC adaption to sprouts may facilitate human infection.

Methods: A STEC reporter strain, created by introducing plasmid pSTEC01 (pSB401backbone with *eae*::luxCDABE) into STEC EDL933, was used to study virulence gene expression during alfalfa seed sprouting. Pesticide-untreated seeds were inoculated with the reporter strain (10^8 CFU/mL), followed by a 12-hour incubation at room temperature. Then, soaked seeds were divided into surface-decontaminated (using 2,000 ppm of $Ca(ClO)_2$), and non-decontaminated groups, then underwent a 5-day sprouting process with regular water sprinkling. After sprouting, STEC populations (internalized and total) were determined, as well as *eae* expression was detected by measuring the luminescence output for both fresh and refrigerated sprouts. RT-qPCR was used to explore a panel of stress response and virulence genes. Data were analyzed in GraphPad software.

Results: During the 5-day alfalfa sprouting, normalized *eae* expression was the highest on day 2, and was significantly higher in non-decontaminated seeds (253.16±6.07 luminescence/(log CFU)) than surface-decontaminated seeds (221.94±4.18 luminescence/(log CFU), $p < 0.0001$). On day 5, non-decontaminated seeds had higher total and internalized STEC levels (8.58±0.04 log CFU/g and 7.72±0.06 log CFU/g, respectively) than decontaminated seeds (8.03±0.14 log CFU/g and 6.47±0.32 log CFU/g, respectively, $p < 0.01$). For refrigerated sprouts on day 8, gene expression analysis revealed upregulation of the stress response gene *SodA* and downregulation of *eae*, *rpoS*, and *espA*. Compared to day 5 (non-refrigerated sprouts), both groups exhibited substantial *eae* and *fliC* downregulation, but had *SodA* upregulated.

Significance: The study underscored that adaptation of STEC to sprouts could increase the pathogen proliferation and this adaptation may modulate gene expression related to stress response and virulence.

T7-04 Change in Microbial Population in Farm Ponds and Irrigation Distribution Systems throughout the 2023 Crop Production Season in Georgia Coastal Plains Area

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Introduction: Biofilms forming inside crop irrigation systems (CIS) may provide a harborage point for foodborne pathogens. Studies on the distribution of microbial populations within CIS are limited.

Purpose: To evaluate the ecology, diversity and composition of the microbial communities present in different CIS in Georgia Coastal Plains area during the 2023 (May-November) growing season.

Methods: DNA samples (n=238) were extracted from HiCap swabs collected from six drip systems and two center pivots (CP) and sequenced for taxonomy determination. Oxford nanopore technologies' SQK-16S024 kit was used for a 48-hour run, followed by bioinformatics analysis using EPI2ME and WIMP. Water samples (n=81) from irrigation source or end of a drip line were tested for total coliforms (TC) and generic *Escherichia coli* (EC) using Colilert with Quanti-tray 2000 MPN trays. Water physicochemical parameters (WPP) were measured within 24 hours of sampling.

Results: Taxonomy analyses were performed with an alpha of 0.5, a minimum accuracy of 80%, and an average quality score of 10.5. Overall, there was a wide diversity of microbial populations within the drip and/or the CP systems. *Bacillus* spp. was abundant in the CP throughout the growing season. *Exiguobacterium*, *Pseudomonas*, *Aeromonas* and *Deinococcus* were abundant in most drip systems throughout the year with an increase in microbial diversity at

the end of the tubing. TC values were similar throughout the sampling period between all farms except for two. EC populations were not different between months or farms (~ 1.0-14.0 CFU/100 mL; $p>0.05$). WPP' influence on TC and EC was dependent on the farm and month of sample collection.

Significance: This work provides a comprehensive evaluation of the distribution of bacterial communities in water and irrigation systems in Georgia coastal plain areas. Foodborne pathogens commonly associated with fresh produce were not isolated. This study will better allow researchers to mimic natural microbial communities in lab settings.

T7-05 Comparison of Modified Washing Machines (Speed Queen & Whirlpool) with Commercial Green Spinners Used for Drying Leafy Greens

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Introduction: This study addresses the microbial risks associated with using modified washing machines for drying fresh produce in small-scale farming operations. The Center for Disease Control highlights contaminated equipment as a significant contributor to foodborne illnesses in food establishments, with smaller-scale farmers often opting for modified washing machines as a cost-effective alternative to commercial green spinners. However, the potential microbial risks of these modified units remain understudied.

Purpose*:* The objective of this research is twofold: first, to compare the microbial quality and safety risks of modified washing machines (Speed Queen TC5000 and Whirlpool WTW5000DW) against a commercial green spinner (Electrolux 600095 VP2) in small-scale operations, and second, to evaluate the cleaning and sanitation efficiency of modified washing machines.

Methods*:* The study involved modifying washing machines following the Washing Machine/Greens Spinner Conversion Guide, with support from the UVM EXTENSION AG ENGINEERING team. *Listeria innocua* was used as a microbial indicator at different concentrations (103 and 106 CFU/ml), and experiments assessed microbial loads before and after various treatments, including postharvest washing and different cleaning and sanitation methods.

Results*:* Results indicate microbial spread on contact surfaces and spinach within the drying units. However, no significant difference was observed in microbial transfer between inoculated spinach and uninoculated green spinners, suggesting that contamination can occur similarly in all three units. Frequent cleaning with Dawn detergent and sanitation (peroxyacetic acid PAA and chlorine) effectively reduced microbial contamination. Additionally, shelf-life studies from days 0–10 were conducted to compare the overall quality against the different drying units.

Significance: This study emphasizes the importance of regular cleaning and sanitation practices to mitigate microbial risks in drying units, irrespective of the type. The results provide insight for small-scale farmers seeking cost-effective alternatives while ensuring the safety of fresh produce.

T7-06 Evaluation of Surface Water Treatment Efficacy Protocol Using Calcium Hypochlorite and PAA against STEC in Open Florida Waters

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❖ *Developing Scientist Entrant*

Introduction: Surface water has been implicated as a source of microbiological contamination for produce. Growers are under market-driven and regulatory pressure to treat surface water before use that contacts produce.

Purpose: This study evaluated the FDA's water treatment efficacy protocol using Florida agricultural water.

Methods: Agricultural waters from a Florida pond and canal were collected, and quality characteristics measured. Samples (98ml) were inoculated with 1ml of a Rifampicin-resistant STEC cocktail (ca. 9 log). Water (99ml) was equilibrated at 12 or 32°C for ≥30min. Calcium hypochlorite (Cl) or PAA was mixed with PBDW to create a stock solution, from which 1ml was added to the 99ml to achieve high and low concentrations of each sanitizer (Cl, 2-4 and 10-12 ppm; PAA, 6 and 10) ppm. Following sanitizer addition, STEC populations were determined at 1, 5, and 10 min by serial dilutions in sodium metabisulfite (28 g/L), plating onto Brain Heart Infusion Agar with rifampicin, and incubating at 35±2°C for 24±2h. Colonies were counted by hand and expressed as log CFU/ml; student t-tests and ANOVA were performed (n=9).

Results: Cl stock solution treatment concentrations ranged from 380-1800 and 600-2800 ppm in pond and canal water, respectively; PAA stock solution treatment concentrations were 500 or 800 ppm consistently in both waters. Low-range Cl treatment in pond water did not achieve a ³3 log reduction in pond water at any contact time but did in canal water after 1min. High-range Cl treatment showed significant ($p<0.01$) reductions in pond and canal water after 1min. Both PAA treatments achieved a ³3 log reduction in pond and canal water within 5min. Reductions may have been impacted by water quality characteristics; population rebounds were observed during longer treatments in both waters.

Significance: Cl and PAA are effective for surface water treatment of STEC for ponds and canals in Florida.

T7-07 Genome-Wide Transcriptomic Responses of *Escherichia coli* O157:H7 Inoculated to Live Romaine Lettuce Followed by Harvesting and Simulated Source or Forward Processing Conditions

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Introduction: Harvested lettuce directed to distant markets is transported via ground shipping to remote processing facilities near destination markets, termed Forward Processing (FP). Source Processing (SP) refers to handling of raw material in facilities close to production areas. How SP or FP conditions affect *Escherichia coli* O157:H7 physiology on lettuce is unknown.

Purpose*:* Evaluate *E. coli* O157:H7 behavior on Romaine lettuce after simulated SP/FP conditions.

Methods*:* Romaine lettuce (cv. 'Rio Bravo') was grown to maturity and potted plants inoculated with *E. coli* O157:H7 EDL933 (reference) or 2705C (outbreak) strains. After 24 hours, plants were harvested and kept at 4°C for 1 day to simulate SP or also under modified atmospheric pressure for 7 days to simulate FP. Before (BP) or after SP/FP, lettuce-associated and fresh culture (FC) bacteria were recovered for RNA extraction and RNA-seq analysis, then analyzed on the Galaxy web platform.

Results*:* Overall gene expression patterns for both *E. coli* strains shifted before and after lettuce inoculation. Transcriptomic responses differed for BP, SP and FP in 2705C, and BP differed from SP/FP in EDL933. When compared to FC, the majority of differentially expressed genes (DEG, $p<0.05$ and $Abs(\log_2(\text{fold change}))\geq 1$) were the same among BP/SP/FP samples for both strains, suggesting that *E. coli* responded more strongly to the shift to the phyllosphere than to the various environmental conditions of SP/FP. Compared to FC, DEG functions in EDL933 before lettuce processing were enriched ($p<0.05$) in, e.g., transport (304), motility (42), chemotaxis (28), while response to stimulus was enriched after SP (50) and FP (305). DEG functions in 2705C were predominantly enriched in response to stress (138), catabolic processes (125) and response to stimulus (36) on BP lettuce, while transport (164/173) and SOS response (16/15) were also enriched after SP/FP, respectively.

Significance: Enriched DEG functions in *E. coli* O157:H7 on lettuce after source and forward processing related to enhanced stress tolerance and reduced chemotaxis and motility.

T7-08 Microbiome Analysis of Packaged Baby Spinach from Controlled Environmental Agriculture and Open Field Production

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Introduction: Consumption of fresh leafy greens grown via controlled environmental agriculture (CEA) has been rapidly growing. However, no information is currently available regarding the microbial community profiles of CEA leafy greens on the market.

Purpose: To assess the microbial profiles of packaged ready-to-eat CEA and open field (OFP) grown baby spinach collected from retail stores.

Methods: Multiple batches of baby spinach from CEA and OFP with similar Best If Used By Dates were purchased from retail stores from Maryland and Florida. Aerobic bacterial count (AC) and yeast and mold (YM) populations were determined. Microbiome was analyzed via high-throughput 16S rRNA gene and ITS amplicon sequencing.

Results: AC and YM vary from trials to trials, but they are generally comparable between CEA and OFP. However, significant difference was observed on microbial community between CEA and OFP. The dominant microbes on OFP products sampled from both sources of OFP were similar, such as bacterial genera of *Pseudomonas* and *Pantoea* and fungal genus of *Cystofilobasidium,* the most abundant bacteria identified on CEA baby spinach in Maryland (grown hydroponically) was a cyanobacteria genus *Synechocystis*, and the major fungal genus on Florida CEA samples was *Papiliotrema*. In general, alpha diversities of both bacterial and fungal communities on CEA baby spinach were higher than OFP products ($p<0.05$).

Significance: To our knowledge, this is the first report of microbial community on CEA leafy greens. Findings, especially the revelation of the large population of cyanobacteria genus *Synechocys* on CEA baby spinach growth hydroponically may point out new research directions regarding the interaction of cyanobacteria and foodborne human pathogens and food safety consequences. Information is also useful for the understanding of algae in CEA environment and transfer to RTE food products grown hydroponically.

T7-09 Survival of *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* Serovars in Microgreens Grown from Contaminated Seeds

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❖ ***Developing Scientist Entrant***

Introduction: The consumption of novel salad greens such as microgreens has increased tremendously around the globe in recent years because of their health and nutritional benefits including the helpful bioactive compounds.

Purpose: In controlled environment agricultural continuums, two critical points of entry of foodborne pathogens have been identified, namely seeds and irrigation water. This study aims to establish the survival trends of these pathogens when microgreens are cultivated using contaminated seeds.

Methods: Seeds of daikon, mustard, broccoli, and red cabbage microgreens were inoculated with a cocktail of two strains of *Salmonella enterica*, *E. coli* O157:H7 or three strains of *Listeria monocytogenes* at low and high inoculum levels and allowed to germinate on soil beds. On days 7 and 14, microgreen and soil samples (n=288) were harvested, pummeled in TSB (5X volume), and plated on XLT4, SMAC and MOX agar. Results from three independent replicates were analyzed for significant differences due to microgreens and contamination level.

Results: Red cabbage, daikon and broccoli showed a decrease of 1 log over 14 days when inoculated with 3 log CFU/g of *Salmonella* compared to 2 log decrease in mustard microgreens. A significant decrease in daikon and mustard was observed when seeds were inoculated at 5 log CFU/g *Salmonella*. Similarly, *E. coli* was significantly decreased in microgreens over 14 days when inoculated at 3 log CFU/g. However, microgreens inoculated with *Listeria* either increased or remained constant when seeds were contaminated with 3 and 5 log CFU/g, respectively.

Significance: Controlled-environment agriculture is helping to manage climate change related uncertainties, wherein resources and environmental conditions can be monitored. The constant recycling of resources allows for pathogens to be recirculated once they enter the controlled environment agricultural continuum and these commodities need a thorough risk assessment to be carried out before they can be sent to retail for human consumption.

T7-10 Survival of *L. monocytogenes* on Waxed Peaches

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❖ ***Developing Scientist Entrant***

Introduction: Fruits such as peaches are coated with wax after hydrocooling and washing. Wax application on fruit surfaces is done to enhance the appearance of the commodity and increase their shelf life. Differences in ability to survive on waxed fruit surfaces can occur among *L. monocytogenes* isolates.

Purpose: In this study, the survival of peach packing house isolate *L. monocytogenes* (*L. monocytogenes* peach) and the caramel apple outbreak isolate (*L. monocytogenes* 573-035) on waxed peaches was evaluated over a 10-days.

Methods: Both the peach and caramel apple outbreak isolates of *L. monocytogenes* were grown on Tryptic Soy Agar with 0.6% yeast extract (TSAYE) at 37ºC for 48h. Suspensions of the cultures were prepared by suspending 2 loopfuls of the culture in 10 ml PBS and vortexing. The suspension (9.02±0.1 CFU/ml) was used to spot inoculate peach surfaces. Peaches were dried in the biohood for a duration of 2h and then sprayed with wax (PrimaFresh 220). After an hour of drying at room temperature, the peaches were stored at 4 ℃ and sampled for enumeration on days 0, 2, 4, 6, 8, 10 on Chromagar (RAPID'L. mono). Colonies were counted after incubation at 37 ℃ for 48h. Three biological and three technical replicates were performed for each experiment.

Results: The initial population (Day 0) *L. monocytogenes* on waxed peaches was 5.71±0.61 log CFU/cm^2. No significant differences were observed between the *L. monocytogenes* peach isolate and caramel apple outbreak isolate on waxed peaches during days 0, 2, 4, 6, 8 ($p\geq0.05$). The population of *L. monocytogenes* (peach) on day 10 was 5.81±0.38 log CFU/cm^2 and was significantly higher than the caramel apple outbreak isolate, 5.12±0.64 log CFU/cm^2 ($p\leq0.05$).

Significance: These results suggest that variations in persistence among *L. monocytogenes* isolates could be observed during prolonged storage of wax coated peaches.

T7-11 Ultrafine Ozone Bubbles Reduce Cross-Contamination with *Listeria monocytogenes*, *Salmonella enterica,* and *Escherichia coli* O157:H7 during Fresh Produce Washing

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❖ ***Developing Scientist Entrant***

Introduction: Water used for fresh produce washing can act as a vehicle to transfer pathogens which leads to foodborne outbreaks in humans. Commercial sanitizers are not completely effective in inactivating the pathogens.

Purpose: The overall goal of this project was to develop novel washing treatments using ultrafine ozone (UFO) bubbles to reduce the cross-contamination with *Listeria monocytogenes*, *Salmonella enterica,* and *Escherichia coli* O157:H7 on cantaloupe and lettuce without affecting the quality and color.

Methods: UFO bubbles were produced using an ozone injected-nanobubble generator system. The generated UFO bubbles were characterized for size, concentration, and oxidation potential. Thereafter, the efficacy of UFO bubbles in reducing the cross-contamination of pathogens was carried out by washing inoculated and non-inoculated produce in bulk for 5 min at 25°C. The surviving pathogens in the wash water were enumerated.

Results: Bubble number in water was approximately 10^8/ml with size ranging from 100-200 nm. The dissolved ozone concentration in UFO bubble water was ~ 8 mg/L at 25°C. UFO at 0.5 mg/L completely inactivated the planktonic cells to below the detection limit as early as 60 sec of treatment. Washing of fresh produce with UFO bubble water significantly reduced the cross-contamination of *L. monocytogenes, S. enterica,* and *E. coli* O157:H7 by 70-90% compared to control, as early as 5 min of treatment time (p<0.05). UFO water inactivated pathogens in wash water by ~ 5 log by 5 min of treatment time, as compared to control (p<0.05). The wash treatment did not affect the color parameters (L, a, b values) of the fresh produce (p>0.05). The effect of UFO wash on the quality of the produce is currently underway.

Significance: UFO bubble water could be used for produce decontamination and prevent cross-contamination without affecting the color of the product.

T7-12 Working Together: Risk Assessment Supporting Risk Management of Frozen Berries Imported into New Zealand

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Introduction: Recent years have seen an increase in outbreaks involving frozen berries worldwide, including the occurrence of two hepatitis A outbreaks in New Zealand in 2015 and 2022. Consumer behaviors are also changing with some food types and practices becoming more popular. This has led New Zealand Food Safety (NZFS) to review the effectiveness of New Zealand's current import controls for frozen berries.

Purpose*:* This presentation discusses the approach taken by NZFS to address the challenges represented by the microbiological risks associated with frozen berries imported into New Zealand. It shows how risk assessment has supported risk management in New Zealand's approach to the setting of new import requirements for frozen berries.

Methods*:* The development of the new import process was conducted in two phases. The first phase involved the assessment of microbiological risks associated with frozen berries. This included a scientific review of outbreak data and information obtained from three surveys on the supply chain and usage of frozen berries by New Zealand household consumers and food service businesses. The second phase focused on gathering information about berry imports, the food businesses involved in their importation, processing and retail sale, and the controls applied throughout the supply chain.

Results*:* Findings indicate that imported frozen berries continue to present a significant risk from norovirus and hepatitis A virus contamination for New Zealand consumers. The two phases supported the identification of risk management options and setting of regulatory controls for the importation of frozen berries into New Zealand. A brief overview of the updated New Zealand import requirements for frozen berries is provided.

Significance: The collective outcomes from the scientific studies and from the discussions between regulators and industry representatives have supported the strengthening of food safety risk management measures for imported frozen berries to protect the health of the New Zealand consumers.

T8-01 A Single Methodology for Honey Authentication

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❖ ***Developing Scientist Entrant***

Introduction: Honey is a popular natural food product, but it is highly susceptible to fraudulent practices such as origin mislabeling or adulteration with inferior syrups, hence robust tests are needed to confirm product authenticity. Recently, molecular methods have been proposed to characterize botanical origin of honey using DNA markers.

Purpose: We hypothesize that DNA markers can be combined to detect both floral sources and exogenous sugars in a single methodology for botanical characterization and sugar adulterant detection in honey.

Methods: Honey samples (224 heather and 28 non-heather) were collected from UK bee farmers in 2021-2022, and corn syrup was purchased online. Specific DNA markers were designed for UK heather species (*Calluna vulgaris* and *Erica cinerea*) and corn (*Zea mays*) and tested for specificity on plant DNA extracts. Furthermore, DNA extractions were prepared from 10g pure honey/syrup or honey spiked with corn syrup at 1-30% (w/w), and the DNA markers were amplified using qPCR. For botanical authentication the amplification (C_q value) of the heather specific markers were compared to that of the general plant marker targeting the conserved *trnL* P6 loop, for semi-quantitative purposes.

Results: DNA markers were used to confirm the presence of heather in honey samples, with 204 heather honey samples showing strong amplification of the marker/s (within 3 cycles of the plant marker), and no significant amplification in the non-heather honey samples. Furthermore, the corn marker was detected in spiked honey samples with a detection limit of 1%, displaying the suitability of the method to detect DNA from both botanical sources and exogenous sugars.

Significance: We demonstrate that novel DNA markers can be combined to create a simple yet robust methodology for honey authentication using one sample preparation. Moreover, the test can be extrapolated to include DNA markers for different plant, animal, or microbial species of interest, and applied to multiple areas of honey authenticity testing.

T8-02 An Investigation into the Impact of Brexit on Consumer Perception of Trust in the Food Industry

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❖ ***Developing Scientist Entrant***

Introduction: This ongoing project investigates the impact of Brexit on consumer perceptions of trust in the food industry. Brexit has significantly impacted the movement of food/agricultural produce, regulations, and cross-border collaborations between Great Britain, Northern Ireland, and the Republic of Ireland. Amid these changes, monitoring the entire food supply chain has become increasingly challenging, heightening the risk of food fraud and safety incidents. As consumers play a pivotal role in shaping the market, understanding any shifts in trust, post-Brexit, enables them to navigate the market with confidence and awareness.

Purpose: This study aims to explore the complexities of consumer perceptions, focusing on trust as a cornerstone of consumer confidence in the post-Brexit food landscape. The objectives include comparing trust in official controls pre- and post-Brexit, determining consumer awareness of food fraud, and devising recommendations that reflect the evidence from this primary research regarding consumer trust in food authenticity post-Brexit.

Methods: The research uses an exploratory sequential mixed methods approach, combining qualitative methods focus groups with a large-scale survey. Sixteen participants were recruited for this study, eight each from both Northern Ireland and the Republic of Ireland. This was to ensure a diverse representation of relevant consumers from the target population. The data from the focus group were transcribed word for word (verbatim) and manually analysed using the thematic analysis method.

Results: Initial results shows that following Brexit, alterations in food labelling, decreased quantities, and changes in food quality have been observed in both Northern Ireland and the Republic of Ireland. This has led to reduced food choices and an increase in food prices, significantly reducing consumer trust levels in both regions. A survey of 1,000 participants will further investigate these impacts.

Significance: The results will inform official controls and consumer-facing messaging contributing valuable insights to navigate the evolving post-Brexit food landscape.

T8-03 Certain Food Matrices May Bind Staphylococcal Toxins Leading to False Negative Laboratory Results

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Introduction: The seven classical toxins of *Staphylococcus* - A, B, C1, C2, C3, D and E - are hazardous to humans and the available detection assays are not reliably sensitive.

Purpose: To improve the detection of classical staphylococcal toxins in food, we developed a new sensitive multiplex enzyme-linked immunosorbent assay (ELISA) and applied it to cheese, milk, mushroom and salmon.

Methods: Toxins (ng quantities) were used to spike food samples and extracted by a process requiring acidification, neutralization and dialysis. The food extract was applied to the ELISA designed with a specific monoclonal antibody to capture each toxin, and a polyclonal antibody and a horseradish peroxidase enzyme-labelled antibody conjugate for detection.

Results: Assay led to a sensitive detection of toxins in cheese at a lower limit of detection of 0.008 – 0.05 ng per g of cheese ($p < 0.05$), except for C2 which was detected at 0.1 ng/g of food. In general, the sensitivity of the new assay met or surpassed the official sensitivity limit set by the food regulatory agencies in Canada and the United States (namely 0.05 ng/g of food) and allowed each toxin to be assessed individually. Toxins present in milk, mushroom and salmon were equally detected at a high sensitivity except that toxin A was not detected in milk, ham or salmon, toxin D not in salmon and toxin E not in ham ($p>0.05$).

Significance: Certain food matrices including milk, mushroom or salmon may bind specific staphylococcal toxins making them unavailable for detection, and thereby generate false negative results leading to consumer vulnerability, hazard incidence under-reporting and an unreliable risk assessment. To protect consumers, instances of problematic toxin-food interactions that hinder hazard detection need to be identified, and the affected food sample subjected to an effective laboratory extraction procedure that would overcome the reduced sensitivity.

T8-04 Detection of Low-Density Foreign Matters in Food Using Sub-Terahertz Wave Imaging System

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❖ ***Developing Scientist Entrant***

Introduction: Foreign matters are defined as any extraneous foreign objects that can contaminate food during production, processing, distribution, and storage. Detecting non-conductive and low-density matters in food has been problematic due to the absence of proper detection methods. Terahertz (THz) technology, first developed in the 1990s, has been advanced for employment in the detection of foreign matters.

Purpose: This study employed a cost-effective sub-THz wave imaging system to detect low-density foreign matters for practical use in the food industry.

Methods: The sub-THz wave was transmitted through a food sample on a petri dish placed on a conveyor belt moving at 30 cm/s. The sample was read by the scanner underneath the conveyor belt, and an image of sample was visualized. After optimizing the attenuator for adjusted sub-THz power, six infant snacks including freeze-dried fruit chip, rice cereal, sweet potato stick, tofu waffle, rice snack, and puffed snack were selected. Foreign matters such as rubber [ethylene-propylene-diene monomer (EPDM)], plastic materials [polyurethane (PE), polyvinyl chloride (PVC)], silicone, and insects (housefly, Indianmeal moth, and cockroach) were placed on, inside, and under several layers of each snack. Finally, the image of each snack with each foreign matter was observed using the sub-THz imaging system under simulated conditions.

Results: The attenuator's value was optimized depending on six popular infant snacks. All tested foreign matters placed above and in between snacks were successfully detected, with the exception of Indianmeal moth underneath puffed snack and freeze-dried chip. However, all tested foreign matters underneath rice snack, rice cereal, tofu waffle, and sweet potato stick were not detected. The detection threshold of the sub-THz wave imaging system was determined to be 3 mm size of PU, PVC, EPDM, and silicone underneath puffed snack and freeze-dried chip.

Significance: This study demonstrated an excellent feasibility of real-time, easy, and practical detection method available in food industry.

P1-145 Hepatitis A Virus Inactivation on Stainless-Steel Surfaces Using UV-C Light Systems Combined with Hydrogen Peroxide

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Introduction: Hepatitis A virus (HAV) outbreaks associated with cross-contamination of food contact surfaces pose significant public health risks. Improved viral inactivation methods include novel ultraviolet light (UV-C) technologies in combination with oxidizing agents.

Purpose: This research aimed to determine the inactivation of HAV on stainless-steel (SS) surfaces using two UV-C (at 254 nm and 279 nm) systems in combination with 3% hydrogen peroxide.

Methods: HAV at ~7 log PFU/mL was aseptically spread on sterile SS coupons (3x3 cm^2), air-dried, and treated with 254 nm-UV-C or 279 nm-UV-C alone, 3% hydrogen peroxide alone, or a combination of UV-C with 3% hydrogen peroxide for up to 1 min. Infectious HAV was recovered from SS coupons in cell-culture media containing 2% fetal bovine serum. Viral titers were determined using plaque assays on confluent FRhK-4 cells within 6-well plates and compared to control. Each experiment was replicated thrice, and data were statistically analyzed using Tukey's adjustment ($p \leq 0.05$).

Results: HAV treated with 279 nm-UV-C showed reductions of 1.18, 1.49 and 2.27 log PFU, while 0.64, 0.94, and 1.28 log PFU reductions were obtained with 3% hydrogen peroxide treatments for 30, 45 and 60 s, respectively. Combined treatments of 279 nm-UV-C together with 3% hydrogen peroxide caused increased HAV reductions of 2.59 to 3.21 log. HAV treatments with 254 nm-UV-C resulted in reduction of 1.38, 2.22, and 2.96 log PFU after 30 s, 45 s and 60 s, respectively. However, treatments of 254 nm-UV-C together with 3% hydrogen peroxide resulted in maximum HAV reductions of 3.12 log.

Significance: Our results indicate that combination of 279 nm-UV-C with 3% hydrogen peroxide could cause increased HAV reduction than individual treatments. However, 254 nm-UV-C treatment showed similar HAV inactivation to combined treatments for the tested times. Hence, UV-C combination with 3% hydrogen peroxide is more applicable using 279 nm-UV-C systems than 254 nm-UV-C systems.

T8-06 Measuring the UV-C Inactivation Kinetics and Determining the Fluences Required for Incremental Inactivation of *Alicyclobacillus acidoterrestris* (AAT) Spores Associated with High-acid Beverage Spoilage

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Introduction: *Alicyclobacillus acidoterrestris* is a spore-forming thermo-acidophilic bacterium that has become a significant concern in the fruit juices industry due to its spoilage potential and mixed resistance nature. Although UV-C irradiation has been successfully applied for pathogen inactivation, its efficacy in inactivating spoilage organisms and extending the shelf life of UV-C-treated beverages is poorly understood.

Purpose: Experimentally measure the UV-C inactivation kinetics and determining the appropriate wavelength (254 vs. 268 nm) and fluence (UV dose) required for incremental log inactivation of AAT spores suspended in PBS.

Methods: Spores from five strains of *Alicyclobacillus acidoterrestris*: ATCC 49025, DSM 2498, VF, SAC and WAC were individually treated with UV-C cumulative doses ranging up to 100 mJ/cm^2 using a collimated beam device emitting UV-C at 254 or 268 nm. Exposure time for each UV dose was calculated using IUVA approved methods. All experiments were performed in triplicate. The log reduction from each treatment was determined using the plate count method and plotted against UV-C doses. Curve fitting was attempted using both linear and non-linear regression models to describe the experimental data, assess model performance, and make comparisons.

Results: Among the five strains, spores of SAC exhibited higher sensitivity, while WAC demonstrated greater resistance than all the other strains studied. Higher inactivation was attained at 268 nm compared to 254 nm. At 254 nm, fluences in the range of 50 - 80 mJ/cm^2 were required to achieve >4-log CFU/mL inactivation of AAT, while at 268 nm, 40 - 60 mJ/cm^2 was sufficient to achieve similar inactivation. The data were well-described by the Weibull model, with R-squared values ranging between 0.87 and 0.98 at both wavelengths.

Significance: This study lays the foundation for determining the UV-C doses required to inactivate AAT spores in liquid foods and designing successful UV-based non-thermal pasteurization systems.

T8-07 Effect of Growth Media on the Pressure Resistance of *Listeria monocytogenes* on Beef and a Plant-Based Meat Alternative

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❖ ***Developing Scientist Entrant***

Introduction: There is increased consumer demand for plant-based meat alternatives and HPP-treated foods, however, the effect of growth media in validation studies assessing safety of HPP-treated foods is poorly addressed.

Purpose: This study investigates the piezotolerance of *L. monocytogenes* grown in different media and inoculated on beef and plant-based meat alternatives.

Methods: WT (6) *L. monocytogenes* strains grown to stationary-phase in BHI and Tryptic Soy Broth without Dextrose (TSB-D) were individually inoculated (~7.0 log CFU/g) in minced beef and a plant-based alternative (10 g). The matrices were vacuum-packed and pressurized at 300 and 400 MPa for 10 min. Biological duplicates were used, and viability was determined by plating onto *Listeria* Chromogenic Agar Base (ALOA) after incubation (37°C, 48 h). Data were analyzed by one-way ANOVA and Tukey's test.

Results: Higher inactivation occurred in meat compared to the plant-based matrix and strain-dependent pre-culturing effects were noted in three strains. Growth in TSB-D rendered FBR16 (0.76, ± 0.14 & BHI; 0.35 ± 0.05) and FBR13 (1.14 ± 0.08, BHI; 0.29 ± 0.12) significantly ($p < 0.05$) more sensitive at 300 MPa in beef, contrary to NCTC 10357 (0.34 ± 014, BHI; 1.60 ± 0.25). Despite the increased inactivation at 400 MPa, this was similar for FBR16 and NCTC 10357, while FBR13 showed comparable results for both media. Interestingly, growth in BHI and inoculation in the plant-based matrix resulted in higher inactivation of FBR16 at 400 MPa (0.84 ± 0.33, BHI;1.57 ± 0.33), while FBR13 and NCTC 10357 were again more sensitive when grown in TSB-D and BHI, respectively. Thus, the pre-culturing effect on the latter was matrix independent.

Significance: This study confirms the importance of the growth medium in *L. monocytogenes* piezotolerance and provides information regarding HPP efficacy in plant-based matrices under mild conditions. The data can be used to improve validation of HPP food applications and decontamination efficiency.

T8-08 Efficacy of Zein-Based Nisin-Loaded Electrospun Nanofibers in Inhibiting Growth of Listeria monocytogenes on Peaches

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Introduction: Peaches have been recently associated with a multistate outbreak as a potentially new vehicle in the transmission of Listeria monocytogenes that can persist and survive through storage/transportation chains. Moreover, minimizing postharvest losses of peaches during retail marketing is challenged by their short shelf life under ideal storage conditions, and susceptibility to rot due their tender texture.

Purpose: This is the first report on fabricating zein-based nanofibers (NFs) loaded with nisin (NS) by uniaxial electrospinning (ES), applied as antilisterial wrapping mats for preserving the quality of yellow peach under abusive storage conditions.

Methods: ES conditions (type of solvent, viscosity, conductivity, feed flow rate, applied voltage, and needle-collector distance) were optimized and the optimum blend was prepared from 50% zein (w/v) using DMF as a solvent. Modified montmorillonite clay (10% w/w of polymer) was dispersed as a nanofiller, nisin was incorporated (50 mg/mL) as a co-protein, followed by chemical crosslinking, characterization/application of the generated NFs.

Results: Compatibility and dispersion of ingredients were confirmed by FTIR/XRD/SEM analyses. Crosslinking using glutaraldehyde significantly ($p < 0.05$) improved the NFs' mechanical (TS=1.23 MPa, EB=5.0%), wettability (99.46°), swellability (90%), thermal (T_{max}=342°C) properties, antioxidant/cytotoxic capacity, porosity, surface area, and simultaneously controlled the in-vitro release of nisin for up to 10 days. Direct wrapping with the optimized biodegradable NFs, eradicated *L. monocytogenes* Scott A and aerobic mesophilic populations after 4 days of storage at 20± 2°C, significantly ($p < 0.05$) reduced moisture/texture loss, delayed browning/total soluble solids/pH change, and maintained the acceptable physical appearance of peach with no apparent signs of rot, shrinkage or fungal decay for 8 days.

Significance: Results highlighted the practical value of the novel zein-based NFs in active food packaging as an effective non-thermal postharvest intervention to inhibit pathogen proliferation and prolong the storage life of peach. The overall research data fills the information gap of implementing antimicrobial electrospun NFs in the field of postharvest pathogen control of fruits.

T8-09 Elucidating the Protective Roles of Desiccation-Related Genes in Shiga-Toxin Producing *Escherichia coli* (STEC) O121 during Storage in Bleached Flour

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Introduction: Persistence of Shiga-toxin producing *Escherichia coli* (STEC) within low-moisture foods has led to several STEC-related outbreaks associated with consumption of raw or undercooked flour products once considered microbiologically safe. Though the molecular mechanisms by which STEC survives in flour are not well understood, transcriptomic data has identified several genes potentially important for flour survival.

Purpose: This study sought to validate previous transcriptome findings by deleting genes and gene networks potentially vital to STEC's ability to persist in flour and assessing the mutant's fitness in flour.

Methods: A chromosomal scarless deletion procedure was optimized for STEC O121 and was used to knock out one of the identified biomarkers, the ProU operon, which encodes a transport system responsible for water retention during osmotic stress. The long-term survival of STEC in flour was compared between the Δ*proU* mutant and its wild-type (WT) by inoculating all-purpose bleached flour at ~8 log CFU/g flour with each strain, replicated six times. Flour samples were collected and enumerated at eight time points within the first month post inoculation.

Results: Following a loss of almost 1 log of culture within the first week after flour inoculation, the WT strain remained at a consistent level of ~7 log CFU/g flour. Similar to previous findings, the WT bacterial population decreased at a much slower rate during storage after an initial ~1 log reduction was reached at 4 days. On the other hand, the Δ*proU* mutant population was reduced by ~1 log within the first 24 hours. Following initial population decrease,

the reduction rate was slowed, similar to WT, indicating the complexity of STEC's persistence mechanisms in flour.

Significance: Functional validation of genes of interest observed from transcriptomic profiling of STEC persistence within flour will increase the understanding of STEC survival in low-moisture environments and help develop new mitigation strategies.

T8-10 Genomic Analysis of *Salmonella* on Wheat Kernels Treated with Lactic Acid over a Six-Month Storage Period

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❖ *Developing Scientist Entrant*

Introduction: *Salmonella* is the most common foodborne pathogen linked to outbreaks of low-moisture foods (LMFs) and studies show that *Salmonella* found in LMFs are highly resistant to inactivation technologies. The molecular mechanisms for such resistance must be elucidated as a first step in the development of effective approaches to control *Salmonella* in LMFs.

Purpose: A longitudinal study was conducted to assess genomic changes that occurred during survival of *Salmonella* Cubana and *Salmonella* Muenchen inoculated on wheat kernels and treated with various lactic acid concentrations, over 6 months.

Methods: Wheat kernels inoculated with *S*. Cubana and *S*. Muenchen were treated with tempering solutions containing 5% Lactic Acid (2mL/Kg and 4mL/Kg). During the 26-week storage period, enumeration was conducted at 2-week intervals. Random colonies from each 2-week sampling interval were selected for whole-genome sequencing (WGS) on an Illumina MiSeq using 300bp paired-end reads. Geneious Prime was used to map the WGS raw reads at each time point to closed parent genomes of *S*. Cubana and *S*. Muenchen.

Results: Over 26 weeks, *Salmonella* populations decreased (3.44 - 3.62 log CFU/g) in lactic acid-treated wheat kernels. Comparative genomic analysis of each isolate to its parent showed numerous SNPs during each sampling time point. Hotspot mutations were discovered in the cell membrane (*bamA* and *bamB*) genes, responsible for the assembly of outer membrane proteins. Additional mutations occurred in metabolic genes, such as *glgA*, involved in glycogen synthesis, indicating additional genomic adaptations during storage.

Significance: Lactic acid treatment reduced the survival of *Salmonella* on wheat kernels. However, *Salmonella* remained viable after 6 months of storage. SNPs that develop in cell membrane genes during storage may increase survival under acid-stress and low moisture-stress conditions. Understanding these changes may lead to the rationale design of inhibitors that could be utilized to control *Salmonella* survival in LMF foods.

T8-11 Non-Thermal Plasma Technology as Mild Processing Technique: Evaluation of the Applicability for Pasteurization of Foods Utilizing Multi-Hollow Surface Dielectric Barrier Discharge (MSDBD) Plasma

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❖ *Developing Scientist Entrant*

Introduction: While the international debate on the safety and quality of ultra-processed foods is taking place, novel pasteurization technologies like non-thermal plasma (NTP) have gained a lot of interest as mild processing techniques.

Purpose: The use of NTP as a pasteurization technique was evaluated towards its antibacterial potential, the effect of various processing parameters, and its effect on different plant-based and meat products with known concentrations.

Methods: A novel Multi-Hollow Surface Dielectric Barrier Discharge (MSDBD) set-up was developed and evaluated for its bactericidal effect on (1) cellulose membrane, (2) agar media enriched with food components and (3) complex food matrices with diverse compositions (self-made sausages, fresh and dried produce, peanut butter, chickpeas, etc.). Samples were inoculated with high levels of *Escherichia coli* O157:H7 and treated with NTP at different doses while maintaining low temperatures. Surviving cells were quantified by standard pour plating.

Results: Operating parameters such as humidity of the input gas (p=0.114) and distance between the sample and the plasma source (p=0.026) were negatively correlated with the bactericidal efficacy of the treatment, while applied energy (function of applied power and treatment time; p=0.002-0.012) and presence of oxygen in the gas mixture (p<0.001) showed a positive correlation. Additionally, it was shown that several food components reduced the bactericidal efficacy of the NTP treatment to a great extent. Lipids (inactivation decreased with at least 2.6 Log CFU), starch (-1.3 Log CFU) or proteins (-1.1 Log CFU) all had a detrimental effect even at low concentrations (4.6, 2.0 & 2.6 m%). As a result, the treatment of food products led to no or very little bacterial reduction.

Significance: This study proves that MSDBD plasma has a direct antibacterial potential, even at low temperatures. However, when applied on foods, the antibacterial effect is very limited due to i.a. nutrients depleting reactive plasma components, reducing possible applications and confirming the need for an *ad hoc* evaluation before use.

T8-12 Pesticide Residue Levels in Tomato Sold in Nairobi Metropolis

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Introduction: Tomato is a nutritious vegetable consumed in meals and processed in various products. However, tomato farming requiring pesticides use for pests and diseases control, pesticides may leave residues in post-harvested tomato.

Purpose: This study sought to determine residues on whole and skins of tomatoes sold in Nairobi.

Methods: The study done from January to June 2017 collected a total of 240 samples. QuECHERS method followed by analysis in a multi-residue standard of 98 residues using GCMS tandem LCMS/MS were used.

Results: About 14 residues were detected during the study; only 9.09 % and 1.51 % of the samples were above EU and Codex MRLs. Residues on skins were significantly more (p <0.05) compared to whole tomatoes. Forty-nine pesticides were detected in whole tomatoes and skins had 10 additional totaling 59 residues. The dry months of January, February and March had more pesticides (n= 6) above EU MRLs compared to wet months of April and May (n=1). Fenamiphos (0.19 mg/kg) and acephate (0.47 mg/kg) levels on the skin were above EU MRLs whereas only dimethomorph (0.03 mg/kg) levels on whole tomatoes were above EU MRL. About 48.5 % of samples had residues of which 27.27 % were single molecules whereas 21.21 % were multiple residues. Single and multiple residues were detected with some above EU and Codex MRLs in dry months. Skins' analysis revealed more residues compared to whole tomato. Some residues not detected in whole tomato were found in skin with some above EU MRLs.

Significance: These presences may be of health concern to consumers and may constitute a public health worry. More studies should be encouraged on fresh vegetables sold in metropolitan cities to control pesticide residues in local markets. Potential improvement requires close monitoring of pesticides by the regulating body to reduce consumers' exposure to chronic ailments related to residues in tomatoes.

T9-01 *Salmonella* spp. and *Listeria monocytogenes* Persistence during Recirculating Hydroponic Cultivation of Leaf Lettuce

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Introduction: Food safety risks associated with hydroponic cultivation of leafy greens have not been well characterized.
Purpose: This study aimed to investigate the persistence and internalization of *Salmonella* Javiana and *Listeria monocytogenes* in recirculating deep water culture (DWC) hydroponic systems during production of lettuce seedlings to mature leaf lettuce heads.
Methods: Recirculating DWC systems containing modified Hoagland's nutrient solution (pH ~5.5) were prepared for cultivation of leaf lettuce. Nutrient solution (NS) in DWC systems containing 25 days old butterhead lettuce (cv. Rex) seedlings were inoculated separately with ~6 log CFU/ml *S.* Javiana (4 trials) or rifampicin resistant *L. monocytogenes* (4 trials). Pathogens were enumerated in NS, rockwool, roots, and leaves with spread plating on day 0 (lettuce seedling), 1, 3, 7, 14, and 21 (mature lettuce head) after inoculation. To lower the limit of detection, membrane filtration was utilized. XLT-4 and BHI supplemented with rifampicin (80 µg/ml) were used for *S.* Javiana and *L. monocytogenes*, respectively.
Results: The initial concentrations of *S.* Javiana and *L. monocytogenes* were 4.10 and 4.62 log CFU/g in roots, 3.15 and 4.19 log CFU/g in rockwool, and 5.86 and 6.66 CFU/ml in NS, respectively. *S.* Javiana persisted in roots, rockwool, and NS throughout the 21 sampling days with a log reduction of 2.85 log CFU/g, 3.66 log CFU/g, and ≥5.50 CFU/ml, respectively. *L. monocytogenes* population declined to <1 log CFU/g in roots, rockwool, and NS between 7 to 14 days. No pathogens were recovered from the edible portion of the lettuce. Pathogen populations in rockwool, roots, and NS were positively associated across sample type (R=0.78 to 0.96). Weak to moderate (R=-0.18 to -0.46) and weak (R=-0.02 to -0.12) associations with pH and temperature, respectively, were calculated for pathogen populations.
Significance: This study indicates that *Salmonella* survival until harvest in DWC systems introduces pathogen transfer risks to leafy greens if not handled appropriately during harvest and post-harvest practices.

T9-02 Boosting Food Safety in LMICs: A Systematic Review of Critical Training Design Factors

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❖ ***Developing Scientist Entrant***

Introduction: Unsafe food in informal markets disproportionately affects low- and middle-income countries (LMICs). Recently there has been much interest & investment in LMIC training programs to improve food safety, however, their effectiveness remains variable.
Purpose: We conducted a multi-vocal systematic review, evaluating peer-reviewed literature and linked- educational content to identify drivers of a successful intervention.
Methods: A systematic search of databases was conducted, and authors of selected studies were contacted for training materials followed by data extraction. Data was extracted on training setting, types of incentives, attractiveness features and trainer characteristics. Incentives were grouped into four categories: economic, social, emotional, and moral. Four attractiveness contributors were defined by existing literature and adapted to LMIC setting. The correlation within attractiveness contributors was tested using phi coefficient.
Results: Twenty-eight publications and the linked training content were taken under review. Only half the studies (n=15) involved pre-testing or needs assessment. The duration and frequency of training sessions was diverse, ranging from one-off 2-hour sessions to multiple full-day sessions. Incentives were used across many studies (n=18) with non-cash economic incentives being the most frequently used. Audio-visual media, likely to boost attractiveness were used in 53% (n=14) studies. A slight but positive correlation emerged between the use of other attractiveness features, i.e. entertaining group activities and participatory problem-solving across trainings (Phi Coeff. = 0.486). Only half of the studies (n=14) mentioned trainer profession, and even fewer disclosed gender or education, raising concerns about trainer capabilities.
Significance: This study uses innovative methods to evaluate food safety trainings in informal markets (multi-vocal review and content analysis). It sheds light on the existing gaps and impact determinants of food safety trainings. There is abundant room for focus on these components and on formative research to identify context-specific training requirements.

T9-03 Do Latin American Consumers Intend to Handle Food Safely to Prevent Foodborne Diseases?

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Introduction: Inadequate food handling in home kitchens often leads to the majority of foodborne diseases outbreaks.
Purpose: To investigate the predictors of Latin American consumers' intention to handle food safely and prevent foodborne diseases.
Methods: Self-administered online questionnaire with 36 items was developed to investigate the Theory of Planned Behavior (TPB) constructs - intention, attitude, subjective norm, perceived behavioral control (PBC), knowledge, risk perception and sociodemographic information. Questionnaire applied to Latin American (LA) consumers had its content validated in Portuguese - Brazil, and Spanish from six countries – Argentina, Chile, Colombia, Ecuador, Mexico and Venezuela, initial focus, by experts and target population. Data were evaluated using partial least squares structural equation modeling. The Brazilian Research Ethics Committee approved the project under the number 5.086.346.
Results: 2341 consumers from 18 LA countries participated with an average age of 35.1 (Standard deviation=13.1). Among the TPB constructs, PBC was the strongest positive predictor (β=0.406; p<0.001) of consumer intention towards safe food handling behavior. This construct is related to the ease or difficulty of performing the behavior, so the consumers recognize the ease of performing safe food handling behaviors. Knowledge and Risk perception significantly influenced all TPB constructs, being stronger on attitude (β=0.336; p<0.005) and PBC (β=0.211; p<0.005), respectively. Both knowledge and risk perception can be influenced by the dissemination of information about the importance of safe handling behavior, in addition to effective risk communication, which implies continuous communication efforts and the use of different methods to identify and understand the target population and their information needs.
Significance: These findings can serve as a basis for risk communication programs focused on consumers in LA, following the recommendations of the Global Strategy for Food Safety proposed by World Health Organization.

T9-04 Consumer Communication Works, But It Goes Slowly: Understanding How Consumers and Experts Evaluate Food Risks

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Introduction: People's perceptions of risks form the basis for their attitudes, intentions, and behaviors. It is crucial for health and risk communication to address the factors that influence these perceptions.

Purpose: This study provides insight into consumers' perception of food-related risks, which, besides technical risk estimates, is essential for designing a clear and targeted health and risk communication strategy. Furthermore its change over time is determined and compared with expert opinions.

Methods: Two surveys were conducted among consumers (n=2005 in 2009 and n=1014 in 2018) and experts (n=62 in 2009 and n=65 in 2018) in the Netherlands to evaluate their familiarity with and perception of food-related risks regarding health, food safety and new technologies.

Results: In both surveys (2009 and 2018) experts ranked unbalanced diet as most important risk followed by food poisoning, and toxins formed during preparation. Consumer risk rankings evolved over the years, aligning more closely towards expert rankings. In 2009 consumers ranked environmental contaminants as main food risk. But in contrast they ranked unbalanced diet as main food risk in 2018, just like the experts. The ranking of food poisoning change from rank 5 to rank 3, also much more in line with experts. Consumers perceived chemical risks and risks associated with new technologies as more significant than experts did. Risk perception increased with age and women perceived risks higher than men.

Significance: A shift towards more awareness of the risk of unbalanced diet and food poisoning by consumers is an important condition in fighting food-related diseases. This study contributes valuable insights into consumers' perceptions of food-related risks, which are important for effective health and risk communication.

T9-05 Do You Need a HACCP Health Check? the Importance of Avoiding Groupthink and Complacency to Really Understand the Effectiveness of Your HACCP System

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Introduction: Most food companies operate HACCP plans within their food safety management systems and HACCP requirements are frequently laid down in legislation and private standards. HACCP assessment forms part of food safety audit but, as only one element of many audit criteria, may not receive the time or expertise needed to evaluate effectiveness. Nevertheless, food company leaders are generally confident that they have done HACCP, but HACCP systems may be operated with a degree of complacency, particularly in food businesses with 'mature' systems.

Purpose: This study aims to promote debate on the need for deeper understanding of HACCP effectiveness within food businesses and discussion of business needs for better HACCP application.

Methods: Requirements of the latest Codex HACCP Principles version (CXC 1-1969; 2022) were reviewed and integrated with research information on HACCP plan effectiveness assessment, HACCP team competency assessment, and interview/focus group research in SME businesses on experiences of HACCP teams in applying HACCP principles and reviewing HACCP plans.

Results: Changes to Codex HACCP included strengthening principles and guidelines on 1. hazard analysis; 2. validation of critical limits and the entire HACCP plan; and 3. verification procedures (including HACCP review). Whilst tools exist for HACCP plan assessment and HACCP team competency, themes from interview and focus group research included HACCP team competency (lack of confidence in ability and accepting the status quo with no challenge – HACCP team groupthink), resource constraints (no time for hazard analysis or review), business culture (HACCP review less important than other business KPIs) and external pressures (audits not challenging HACCP enough, i.e., no pressure to focus).

Significance: Findings reveal a mismatch between HACCP requirements and business HACCP practices, highlighting risks of relying on systems where there is limited challenge of their effectiveness. Further research is needed on interventions to help companies overcome these hurdles and strengthen their food safety management approaches.

T9-06 Exploring Home Food Preservation in Montana: A Quantitative Survey on Practices and Motivations among Home Food Preservers

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Introduction: Foodborne illness, especially dangerous from preserved foods, can be greatly mitigated with research-based food safety practices. Montana is a rural state with diverse agriculture and home food preservation interest is widespread.

Purpose: To explore the local sustainability of home food preservation and assess continued food safety concerns.

Methods: A quantitative Qualtrics survey inquired into home food preservation current practices and future engagement motivators of Montana residents. The survey was distributed through snowball sampling through student and community partners. Data were collected under IRB ID 2023-833-EXEMPT.

Results: A total of 332 responses were received. Of the respondents, 51.8% had some or extensive canning preservation experience in 2022-23. The most frequent reasons for preserving were to save money (48.2%), know where food comes from (42.5%), and/or reduce food waste (42.8%). Preservers fell into four clusters, V*ery limited consumer/preserver (1; 10.5%), Sometimes consumers/limited preserver (2; 23.2%), Sometimes consumer/preserver (3; 20.5%),* and *Frequent consumers/preservers (4; 38.6%)*. Clusters were statistically significantly correlated (p≤.05) with some demographics and beliefs. *Frequent consumers/preservers* were more likely to be rural, older, trust Extension, and see canning as increasing access. Receiving information via social media ranked highest for *cluster 1* (42.2%), short social media videos for *cluster 2 (55.8%),* email for *cluster 3* (53%), and by mail for *cluster 4* (39.4%). In-person workshops were still of interest with 32-46% of interest across clusters, with *cluster 2* with limited experience indicating the most interest.

Significance: This pilot study highlights a great variation in needs and interests, indicating that diverse delivery methods may be needed to meet Montana consumers' needs. This survey tool will be used in an upcoming national, randomized replication survey. To address the resurgence of home food perseveration and prevent botulism outbreaks at home, it is imperative to understand consumer's needs and provide outreach resources and research-based recommendations.

T9-07 Food Safety Culture Enhancement: Intervention Implementation in a Case Study with Pre- and Post-Assessment Comparison

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❖ ***Developing Scientist Entrant***

Introduction: Because of increasingly stringent private certification schemes and legislation, companies are searching for ways to mature their food safety culture.

Purpose: This study provides an overview of the food safety culture improvement journey of a case-study, demonstrating the improvement in a pre-/post-assessment comparison.

Methods: A case study (food processing company, 80 employees, producing prepared meals and soups in Belgium), acted as a living lab for this research. First, a thorough food safety culture assessment was executed, following a validated mixed-method methodology. Next, based on a gap analysis, the management team decided the first focus of improvement, namely the dimension commitment. To improve this first selected gap in food safety culture maturity, an intervention was selected from a previously developed food safety culture intervention portfolio (developed based on scoping reviews and stakeholder consultations). Based on available intervention research and change theory, an eight-step implementation plan was developed and followed in the case study. Six months after implementation, the post-assessment was conducted, which was an exact copy of the pre-assessment.

Results: Pre-/post-assessment comparison showed both an objective (assessment tools like observations) and subjective (through the tools assessing perceptions of employees) improvement of the targeted dimension (i.e. commitment). Furthermore, combining all assessment tools, the targeted dimension underwent the biggest positive change compared to all other dimensions assessed. Furthermore, in general, a positive change was revealed for each included dimension (i.e. leadership, communication, commitment, risk awareness and resources, adaptability, consistency, beliefs & values, and mission, vision, strategy). After data integration, the case study went from having five gaps to having three gaps. In the next improvement cycle, remaining gaps can be selected to improve.

Significance: An effective path to maturing food safety culture was demonstrated. Industry practitioners and academics can use the described methodology to further advance food safety levels across the globe.

T9-08 Hands-On Sanitation Programming Development for Small Processors

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Introduction: Lack of established hands-on sanitation programs have been linked to sanitation non-conformances. There are limited hands-on trainings that are accessible and affordable to small processors. Though current research indicates targeted food safety training positively impacts knowledge of food handlers, industry experts report that it does not significantly impact prolonged sanitary practices. Efforts have been made to identify sanitation job-tasks that small food processors face, so that effective and relevant hands-on training can be piloted and developed.

Purpose: Identify further training needs of small processors to guide revisions of sanitation hands-on training.

Methods: During our 3 pilot in-person sanitation trainings (1 in NC, 2 in MA), surveys (pre- and post-program) and focus groups (post-program) were conducted with ~45 sanitation managers, business owners, and line operators. Surveys asked processors to rank their interest and preparedness in sanitation-related topics on a scale from 1 (no interest or preparedness), to 5 (high interest and preparedness). Focus groups consisted of ~10-15 questions; processors were asked to discuss the courses' uniqueness, rigor, and adoptability. Focus group responses were coded to quantify themes.

Results: Survey results across three cohorts indicated an overall significant increase in interest and preparedness from pre- to post-program. Focus group results indicated that future program opportunities should include dry cleaning, and examples of more robust utensils rather than flat surfaces. Practitioners ranked the courses' uniqueness at 4.5 out of 5, due to camaraderie between participants, swabbing examples, and titration demonstrations. The program's rigor ranged; some said that the course was "a good reminder of proper techniques", while others would have preferred a more "intensive" program. All participants noted that the hands-on program helped them develop "confidence".

Significance: Results imply that hands-on sanitation training programs, even at basic levels, can be a tool for small practitioners to develop preparedness in sanitation skills.

T9-09 Identifying and Addressing Food Safety Inequities in Missouri: A Salmonella Use-Case Using SENS-D, a Sensor-Enabled Decision Support System.

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Introduction: Salmonellosis has not declined in three decades in the United States, and disproportionately impacts vulnerable populations. Technologies that identify population vulnerabilities will be important to better address root causes of food safety disparities through multisector partnerships.

Purpose: Using SENS-D, a sensor-enabled decision support system, we examine Missouri county-level data as a use-case to determine the geospatial disparities for populations facing food insecurity and a disproportionate burden of salmonellosis. In addition, we provide a framework for engaging multisectoral health and supply chain partnerships to address disparities with SENS-D.

Methods: We used a three-phase human-centered design to determine One Health data priorities and decision support needed to mitigate food disparities. Multisectoral, multisource data that were publicly available were geospatially harmonized at the state- and county-levels into a geographic information system. Annual population adjusted salmonellosis rates were calculated using CDC PulseNet data. A quantile analysis was performed to identify geospatial disparities in salmonellosis rates and identify population vulnerabilities. We produced geospatial visualizations to understand population vulnerabilities and food insecurities cooccurring with high salmonellosis rates.

Results: 17.5% of counties (20/114) in Missouri had the highest *Salmonella* rates and food insecurity in 2021. All 20 high risk counties were rural (20/99 rural counties; 20%) and none were urban (0/16 urban counties). There are geospatial inequities with the highest geographic distribution in the southeast region of the state, which coincides with counties with the highest social vulnerabilities (e.g. poverty, lower levels of education, healthcare shortages, etc.).

Significance: Understanding social vulnerabilities and food access can potentially enhance multisector food safety efforts, including health systems, Extension educators, supply chains, and food banks. This preliminary work demonstrates there is value in combining food insecurity with *Salmonella* risks and food-borne illness to strengthen workforce planning and outreach efforts, and work toward early identification of foodborne pathogens in vulnerable populations.

T9-10 Identifying Gaps and Opportunities in Home Food Preservation: A Needs Assessment Focusing on Extension Professionals

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Introduction: The COVID-19 pandemic ignited increased consumer demand for home food preservation (HFP) methods, emphasizing the need for safe, science-based information. In the United States, the National Center for Home Food Preservation observed a 620% increase in website access from March 2020 to 2022.

Purpose: To determine gaps in home food preservation resources and programmatic content used by Extension professionals to support and train their local communities.

Methods: A Qualtrics-based online survey (UGA-IRB ID PROJECT00007664) was distributed via email from Food Safety Extension Network (FSEN) members to their state Extension HFP educators, as well as at the annual National Extension Association of Family and Consumer Sciences (NEAFCS) conference during HFP sessions. The 10-15 minute survey included 25 questions about agent confidence in addressing consumer requests, HFP education in their community, teaching resources, and Specialist support across HFP domains.

Results: Anonymous responses from 141 Extension professionals participating in the FSEN in the Mid-Atlantic and Southern US were recorded, and

65% of respondents had at least 5 years of Extension experience. Findings provided crucial insights into Extension HFP educators' needs. Low numbers of respondents felt they had adequate education in the areas of curing and smoking (3%), freeze drying (4.6%), new HFP equipment/technology (16.9%), and fermentation (21%). These same four areas were also highlighted as gaps in available curricula, Extension classes offered, and low confidence in answering consumer questions.

Significance: Experienced Extension professionals are looking for training and resources to meet community interests in the rapidly evolving area of the science of food preservation beyond canning and freezing. Various states working together to pool resources could minimize this gap and ensure that consumers have access to accurate, science based HFP food safety information. These findings will be used to prioritize the development of resources and training for Extension professionals.

T9-11 Immersive Education: Results and Lessons Learned from Incorporating Innovative Technologies into Food Safety Education Efforts

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Introduction: Incorporating technology into food safety education programs creates opportunities to develop increasingly engaging, effective, and impactful learning scenarios that can reach a broad audience.

Purpose: The purpose of this project is to develop and implement immersive and interactive food safety education tools for owners and operators of small- to mid-sized diversified farms, beginning farmers, socially disadvantaged farmers, extension educators, farmers market vendors, and farmers market managers to increase a participant's general understanding of the presented topics and overall food safety practices through an in-person, instructor-led immersive experience that supplements traditional classroom training.

Methods: Virtual tours of a farmers' market and a small, diversified farm were developed by recording 360° videos which were uploaded into Oculus headsets and edited to include interactive pop-up boxes with food safety information, additional embedded videos, and quizzes to test food safety knowledge; companion instructor guides were also created for each tour. Knowledge assessments, participant evaluations, and fidelity assessments have been used to measure the impact and efficacy of using the headsets as part of an educational program.

Results: The headsets have been incorporated into seven trainings in Virginia and North Carolina to date, reaching 36 Extension educators and 19 farmers/producers/market managers. Reception to the use of the headsets has been positive overall, with participants enjoying the immersive aspects of the training. However, as expected, some users experienced technical difficulties as a result of being unfamiliar with the technology and/or issues with the headsets themselves. Extension educators expressed interest in bringing the headsets to their counties, which has continued through 2024

Significance: The results of this project serve as a case study into the effectiveness of how to develop and incorporate immersive content into food safety education programs, and also challenges to be addressed when using such technologies in the future.

T9-12 Understanding the Food Safety Needs of Small and Very Small Processors in the Northeast United States: Food Safety Communicator and Regulator Perspectives

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Introduction: Lack of awareness of regulatory requirements, knowledge of food safety risks, and perceived costs of implementation have previously been identified as barriers to implementation of HACCP regulations for smaller sized food businesses. Members of the Northeast Center to Advance Food Safety (NECAFS) Preventive Controls (PC) Workgroup explored challenges that small and very small (SVS) food processors face for implementation of requirements in FDA's Current Good Manufacturing Practices, Hazard Analysis, and Risk-Based Preventive Controls for Human Food (PCHF) Rule.

Purpose: The aim of this study was to understand challenges faced by SVS processors covered under the PCHF Rule and to make recommendations for outreach strategies to improve compliance.

Methods: Food safety communicators (FSC) (educators, consultants, and individuals in allied industries) and Regulators located in 12 states in the northeast U.S. were surveyed to determine their perceptions of challenges faced by SVS processors with respect to awareness of the PCHF Rule, knowledge of risk-based approaches to identify and control food safety hazards, and cost and time constraints that present motivational hurdles. An online survey was developed and sent by email to the target audience.

Results: FSC (n=46) and Regulators (n=37) agreed that there are gaps in processor awareness of the PCHF Rule, knowledge of Good Manufacturing Practices (GMPs), sanitation standards, and core elements of the risk-based food safety approaches including conducting a hazard analysis and establishing verification and validation procedures. The PC Workgroup recommended developing outreach activities and clear, concise, learning materials on identified challenge topics that are tailored to SVS processors' cost and time constraints and their specific needs and learning preferences.

Significance: Outreach efforts to increase compliance with the PCHF Rule are an important complement to regulatory enforcement and will provide SVS processors with the tools they need to understand and implement the requirements in the regulation.

T10-01 Assessing Demand for Food Safety in Traditional Markets: Findings from a Baseline Assessment in Ethiopia

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Introduction: Traditional markets in low- and middle-income countries (LMICs) experience unique challenges including inadequate infrastructure and little to no regulatory oversight – all of which can increase food safety risks.

Purpose: This baseline study illustrates the application of novel survey tools developed by the EatSafe program to measure behaviors and behavior drivers relevant to food safety, in south-western Ethiopian markets.

Methods: A total of 460 market consumers and 466 vendors of kale, lettuce, and tomatoes were surveyed from two markets in south-western Ethiopia between April-May 2023. Survey tools were designed around indexes of salience of food safety, self-efficacy in implementing food safety practices, knowledge of food safety principles, and carrying out food safety practices. Food vending and hygiene practices were observed in a subset of vendors (n=214).

Results: Consumers and vendors considered food safety as important, compared to other food choice drivers (average salience index: 69%; SD: 24%). Both groups showed high levels of general food safety knowledge (average knowledge index: 67%; SD: 14%). However, they were less confident about being able to implement food safety practices, as evidenced by moderate self-efficacy scores (average: 53%; SD: 21%). Also, neither group enacted food safety practices often, with the behavior index only reaching an average of 40% (SD: 12%). Differences in self-efficacy and behaviors were observed between men and women, although minor. Behaviors tested included using food safety-related cues to decide which shops to purchase from (consumers), implementing food safety practices at their shop (vendors), and communicating about food safety (consumers and vendors). The tools, first piloted in Nigeria, proved applicable to the Ethiopian context with only minor adaptations.

Significance: This results framework and associated surveys provide tools for programs seeking to leverage consumer demand to improve food safety in traditional markets.

T10-02 Analysis of Seasonal Trends in Viruses That Cause Food and Waterborne Illness Using Wastewater-Based Epidemiology

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❖ ***Developing Scientist Entrant***

Introduction: The true prevalence of enteric viruses that cause food and waterborne illness in our communities is severely underreported – wastewater surveillance can address this disparity.

Purpose: This study aimed to elucidate the seasonal trends in the prevalence and diversity of common food and waterborne enteric viruses in Ontario wastewater.

Methods: Biweekly raw influent wastewater samples were processed from August 31st 2022 – December 25th 2023 from two wastewater treatment plants in Southwestern Ontario (n = 268). Following sample concentration with PEG flocculation and centrifugation, human adenovirus (HAdV-41), norovirus (HNV-GII), rotavirus (RVA), and Hepatitis A, were quantified by qPCR. Samples from each meteorological season with low C_t values for HNV-GII and HAdV-41 were selected for metagenomic sequencing. The Illumina Viral Surveillance Panel was used for target enrichment before sequencing using the MiSeq platform. The data followed a non-parametric distribution so a Wilcoxon Rank-Sum test was used to evaluate seasonality. A cross-correlation function was used to evaluate the Pearson correlation coefficient between HAV clinical and wastewater data.

Results: HAdV-41, HNV-GII, and RVA were detected in every sample, whereas HAV had positive detection in 15 samples and was correlated with clinical cases in one sewershed with a 17-day lead time (p<0.05). HNV-GII and RVA had statistically significant seasonality with peaks in the winter and spring, respectively (p<0.05). HAdV-41 had the greatest concentration in wastewater in autumn of 2023 (p<0.05) but peaked later in the season in 2024 – similar to the major and minor seasonal periods observed in a clinical population. Ratios of HNV genogroups and HAdV serotypes were compared across seasons to complement limited available clinical data.

Significance: Globally, food and waterborne viruses cause considerable morbidity and mortality. Understanding the seasonal trends in their incidence can help public health officials mitigate their spread. These findings can also inform future ongoing wastewater surveillance programs and contribute surveillance data for a group of pathogens that are non-reportable.

T10-03 Brucellosis in Food Animals: Re-Emerging Zoonotic Threat

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Introduction: Brucellosis is a significantly neglected zoonotic disease endemic in many countries. It affects a wide range of various food animal species, leading to economic losses due to the customary sacrifice of infected animals. The leading causes of human infection are consumption of unpasteurized milk or meat or contact with infected animals.

Purpose*:* To assess the seroprevalence and identify risk factors associated with brucellosis in food animals in Qatar. Additionally, the study aims to explore demographic characteristics, prevalent symptoms, causative species, antibiotic susceptibility, and risk factors related to human brucellosis.

Methods*:* 248 camel, 246 sheep, 246 goat blood samples, and 196 human blood culture samples have been collected. Rose Bengal test and competitive-enzyme-linked immunosorbent assay were performed to determine animal brucellosis seroprevalence. The polymerase chain reaction was used to identify *Brucella spp*. E-test was used to investigate antibiotic susceptibility against 8 antibiotics. Regression models were used to perform univariable and multivariable risk factors analysis for both animal and human brucellosis.

Results*:* Seroprevalence was 20.6%, 16.7%, and 2.4% in camels, sheep, and goats, respectively. Univariable analysis showed that old age and the female in camels and young age in sheep were associated with a higher prevalence of brucellosis. Consumption of raw milk was the leading cause of human brucellosis (58.6%). Most human patients were adults (74%), mainly males (80.6%). South Asian patients constituted a significant percentage (42.9%), followed by Qatari patients (28.6%). The most common symptom was fever (91.9%). The causative agent in all patients was *B. melitensis,* and none of the samples showed antibiotic resistance.

Significance: The alarming prevalence of brucellosis in animals, particularly camels and sheep, underscores the urgency for disease control in food animals to mitigate its transmission to humans. Implementing measures that raise awareness about the risks associated with consuming raw milk is imperative.

T10-04 Consumption Frequency and Preparation Practices for Stuffed Breaded Chicken Products – Minnesota, 2023

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Introduction: Not ready-to-eat frozen breaded, stuffed chicken products have been implicated in several *Salmonella* outbreaks, leading to the proposal of declaring *Salmonella* an adulterant in these products in 2023. These products contain cooking instructions to ensure food safety, but little is known about how these products are prepared by consumers and how often.

Purpose*:* Our objective was to characterize frozen breaded stuffed chicken product consumption and preparation practices of Minnesota residents.

Methods*:* We conducted a survey at the 2023 Minnesota State Fair about consumption frequency and preparation practices for frozen foods, appliances owned, and demographic factors. Descriptive statistics were computed to characterize consumption and preparation practices of frozen breaded, stuffed chicken products across demographic factors. We also used descriptive statistics to compare the frequency of following cooking directions with appliances used and point-of-sale status, including raw or cooked.

Results*:* Among 1,719 respondents, 8.6% (n=148) indicated consuming frozen breaded, stuffed chicken products. Frozen breaded, stuffed chicken product consumption was lowest among the youngest respondents, aged 18-24 (6/139, 4.3%), and highest among respondents aged 25-44 (38/409, 9.3%). Most (121/148, 81.8%) respondents indicated always following cooking directions on the packaging and 71.7% (86/121) of whom prepared the products most often in a conventional oven. Among those that reported purchasing these products fully cooked, 88.6% (39/44) reported always following directions, compared to 96.1% (74/77) and 100% (8/8) of those who purchase them uncooked/raw or unknown condition. Those who reported cooking these products on the stove and in an air fryer also reported always following cooking instructions (6/6 and 10/10, respectively), whereas 81.8% (9/11) of microwave users and 95.6% (86/90) conventional oven users reported always following instructions.

Significance: These findings highlight high adherence to packaging directions among those purchasing raw products and changing cooking methods, including the use of air fryers, for these products, which has started to be incorporated into package instructions.

T10-05 Detection of Beverage Spoilage Microorganisms by a Combination of Metabarcoding and PCR Technologies

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Introduction. Alcoholic and non-alcoholic beverages can be contaminated by different spoilage organisms, requiring numerous assays for detection, presenting challenges to diagnostic laboratories in testing and processors for quality assurance management. Streamlined detection of all spoilers is desired to ensure product quality and safety.

Purpose. To establish and evaluate a combined metabarcoding and PCR approach for comprehensive detection of beverage spoilers.

Methods. DNA was amplified by PCR using newly designed primers targeting 16S and 18S rRNA genes of bacteria and yeasts respectively, and published primers for detection of *hor*, *bsr*, and STA1 genes. The 16S and 18S amplicons were sequenced using an illumina MiSeq sequencer. Sequences were analyzed using BaseSpace or Geneious software to identify species based on Greengenes, RDP and custom databases.

Results. The method specificity was tested using 53 DNA samples of 32 microbial species, including *Bacillus, Bifidobacterium, Brettanomyces, Lactobacillus, Pectinatus, Pediococcus*, *Pseudomonas, Saccharomyces* and *Streptococcus* species and 8 plant species. All of the assays detected their respective targets but not the non-targets. The limit of detection of the method was 10^4 cells/mL for beer, wort and fermenter products, and 10^5 cells/mL for yeast slurry as evaluated using samples spiked with 6 bacterial and 3 yeast strains. An enrichment protocol was evaluated using samples spiked with 5 species of *Lactobacillus, Pectinatus* and *Saccharomyces* at 10 CFU/mL and incubated for 48 hours. All targets were successfully detected in beer and wort samples after the enrichment while fractional detection was achieved in yeast slurry and fermenter products likely due to matrix effect. The method was reproducible as evaluated using replicate samples within and between experiments and between analysts.

Significance. The combined metabarcoding and PCR method can be used to detect numerous bacterial and yeast spoilers simultaneously to facilitate quality and safety control measures in production of alcoholic and non-alcoholic beverages.

T1-06 Preliminary Trends in Reported Human Salmonellosis Cases in Virginia, USA Between 2012–2022

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❖ ***Developing Scientist Entrant***

Introduction: Understanding trends in human illnesses, such as salmonellosis, is critical for monitoring progress in reducing foodborne illness burden and understanding the impact of food safety interventions and policy on disease reduction.

Purpose: Analyze passive surveillance data to describe trends in reported human clinical salmonellosis cases in Virginia from 2012-2022.

Methods: Data (representing 12,589 cases) were obtained from the Virginia Department of Health in 2023. Incidence rates per 100,000 (IR) were calculated for five periods: (i) pre-culture independent diagnostic tests adoption (CIDT; 2012-2014), (ii) early CIDT adoption (2015-2016), (iii) late CIDT adoption (2017-2019), (iv) COVID-19 pandemic (2020-2021), and (v) post-pandemic (2022). Incidence rate ratios (IRR) were used to compare IR between each time period.

Results: The state-level IR during 2012-2022 ranged between 11.2 and 15.5, and peaked in 2019 (IR= 15.5), while county-level IR ranged between 2.3 and 132.5 (median= 16.6). IR among persons >17 years old during the late CIDT period (IRR= 1.2; 95% CI=1.1-1.2) was higher than the pre-CIDT era, while IR during the COVID-19 pandemic (IRR= 0.9; 95% CI=0.9-1.0) was not. Unlike previous analyses which suggest a return to pre-pandemic IR for foodborne diseases in 2022, our findings suggest that salmonellosis IR post-pandemic had not returned to pre-pandemic levels in Virginia (IRR for 2022 versus 2017-2019=0.9; 95% CI= 0.8-0.9). From 2012-2022, the highest proportion of cases were reported between July-September (0.4). The most prevalent serotypes reported were *S.* Enteritidis, *S.* Typhimurium/I 4,[5],12:i:-, *S.* Javiana, and *S.* Newport.

Significance: Our study highlights the impact of increased CIDT adoption on reported salmonellosis IR and suggests that in contrast to states within the Foodborne Diseases Active Surveillance Network, the IR for salmonellosis cases in Virginia had not returned to pre-pandemic levels in 2022. Further investigation to determine how increased CIDT use is affecting salmonellosis IR in Virginia will be important for informing public health strategies.

T10-07 Exploring Microbial Spoilage and Pathogen Growth in Plant and Meat-Based Burgers

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Introduction: Plant based burgers are products that may ease the transition from a meat based to a vegetarian-based diet, not requiring changes in the dinner preparation and eating habits and being socially acceptable.

Purpose: The present study aimed to characterize the growth of pathogens and spoilage bacteria in plant based burgers and evaluate corresponding sensory changes and liking.

Methods: The microbiome development and growth *Salmonella*, *Listeria monocytogenes* or *Bacillus cereus* was determined in four plant based burgers (three different batches) using selective agars. A descriptive sensory analysis (trained panel, 10 judges) and a consumer evaluation (N=82, liking) was performed. The burgers were analyzed after 7 days storage at 12 °C. The microbiome was determined by identification of colonies by MALDI-TOF MS and MiSeq (16S rRNA gene).

Results: The initial numbers of bacteria in the burgers were in the range 3.6 [0.1] (mean and standard error of the mean) to 5.1 [0.3] log CFU/gram. During storage, the microbial levels increased to 7.9 [0.08] to 10.6 [0.1] log cfu/gram. The highest growth of inoculated pathogens was found in the burger with lowest initial background microbiota, reaching more than 8 log cfu/gram (inoculum 3.6 [0.3] log CFU/gram). The dominant bacterium was *Bacillus* spp. initially and after storage. Among all burgers, it scored highest on liking among consumers and storage did not affect liking and sensorial profile significantly (p>0.05). Lowest pathogen growth was found in those with highest initial and final background microbiota. These burgers contained a diverse microbiota initially and were dominated by *Pseudomonas* in combination or not with lactic acid bacteria at the end of storage.

Significance: For plant-based burgers, growth of *Bacillus cereus* could be of health importance since growth may be supported during storage at abuse temperatures. Consumers perceive vegetable products as more safe and should be warned that heat stable toxins may be produced in these products if stored at abuse temperatures.

T10-08 Sequelae of Foodborne Infections in British Columbia (BC), Canada, 2005–2014

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Introduction: One in eight Canadians experiences a foodborne infection (FBI) annually. Sequelae and mortality risks are unclear.

Purpose: We assessed the risks of kidney, gastrointestinal, and rheumatological sequelae and mortality following 13 FBIs in British Columbia (BC), Canada.

Methods: We conducted a retrospective cohort study of everyone registered in BC's health insurance program, 2005-2014 (n=5,819,344). FBIs were identified as new laboratory confirmed, provincially reported infections. Sequelae and deaths were identified using administrative data algorithms. We estimated risks using adjusted hazard ratios (aHRs) from extended Cox regression models, adjusting for age, sex, comorbidities, and neighbourhood income. Population attributable fractions (PAFs) were calculated using aHRs.

Results: The cohort was followed for ~7.5 years/person; 40,523 individuals experienced 42,308 FBIs. Compared to those without any FBIs, the risk of HUS was astronomically higher in individuals with STEC in the 1-45 days prior (aHR: 3721; 95% CI: 2443, 5668), while the risk of acute kidney injury was 30.7 times higher (95%CI: 27.4, 34.5) in individuals with *Campylobacter*, hepatitis A, *Salmonella* (non-typhoidal, Paratyphi, Typhi), STEC, *Shigella*, or *Yersinia* infection in the 90 days prior. The risks of inflammatory bowel disease (aHR: 4.12, 95%CI: 3.55, 4.78), celiac disease (aHR: 3.86; 95%CI: 3.32, 4.50), and irritable bowel syndrome (aHR: 4.27, 95%CI: 3.89, 4.69) were significantly higher in individuals with a new FBI at least 6 months prior. Similar results were observed for ankylosing spondylitis, reactive arthritis, and anterior uveitis. The risk of dying in the 30 days following a FBI was highest for *Listeria* (aHR: 90.5; 95%CI: 62.1, 132), followed by STEC (aHR: 10.2; 95%CI: 4.99, 21.1). PAFs were most pronounced for HUS post-STEC, with 29% of HUS in the cohort attributable to laboratory-confirmed, provincially-reported STEC infection.

Significance: Understanding the impacts of FBIs beyond acute gastroenteritis is important to prioritize prevention activities.

T10-09 From Anecdotal to Analytical: Correlating Self-Reported Norovirus-Like Illness with NoroSTAT Data

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Introduction: Human norovirus is the leading cause of foodborne illness in the U.S., with hallmark symptoms of vomiting and diarrhea. The Norovirus Sentinel Testing and Tracking network (NoroSTAT) is a collaborative network of select state public health departments and the U.S. CDC, designed to collect epidemiologic and laboratory data on suspected or confirmed norovirus outbreaks. The crowdsourcing website iwaspoisoned.com (IWP), established in 2009, provides a means by which the public can self-report foodborne disease-like symptoms in real-time. The value of crowdsourced data in relation, or complementary, to classic epidemiologic approaches, is largely unknown.

Purpose: To investigate correlations between self-reported norovirus-like illness from IWP and norovirus epidemiological data from NoroSTAT.

Methods: The analysis covered reports between August, 2018 and May, 2022. NoroSTAT data were obtained from their website (https://www.cdc.gov/norovirus/reporting/norostat/index.html) and internet archives (https://archive.org). Data were extracted directly from the IWP database, with the following inclusion criteria: (i) symptoms of both vomiting and diarrhea reported together (used as a proxy for norovirus-like illness); and (ii) reports originating from a NoroSTAT-participating state. Reports were excluded if spam or duplicate. Associations were investigated using Pearson Correlation and Granger Causality tests using the Minitab (State College, PA) software package.

Results: The Pearson Correlation value of 0.669 was highly significant ($p<0.001$), indicating a strong linear relationship between IWP and NoroSTAT dataset outcomes (those being time and number of reports/cases). The Granger Causality analysis indicated a highly significant directional correlation between the two datasets ($p=0.003$).

Significance: There was a strong positive correlation between self-reported norovirus-like illness trends reported to the IWP crowdsourcing website, and NoroSTAT-reported outbreaks over the same timeframe. The alignment of the crowdsourced symptom data with public health surveillance lends credibility to the value of curated self-reporting as a complementary signal for detecting and perhaps predicting emerging norovirus epidemiological reports and/or trends over time.

T10-10 Stricken with Chicken: The History of Multi-Jurisdictional *Salmonella* Clusters Related to Poultry in Canada from May 2017 to December 2023

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Introduction: Following the implementation of whole genome sequencing (WGS) as the primary method of typing *Salmonella* isolates in Canada in 2017, a significant number of *Salmonella* clusters associated with poultry products were detected and investigated.

Purpose: To summarize the evidence for fresh and frozen poultry products as an ongoing vehicle of infection for multi-jurisdictional *Salmonella* clusters in Canada.

Methods: Human and non-human *Salmonella* isolates were sequenced at national and provincial laboratories and compared nationally by PulseNet Canada using whole genome multi-locus sequencing typing (wgMLST). Cluster codes were assigned when 2 or more cases grouped together within 10 wgMLST allele differences within 60 days; a higher threshold was used for more common *Salmonella* serotypes. Case exposure data were collected from provincial/territorial health authorities for select WGS clusters to determine the vehicle of infection, and outbreak investigations were initiated when required. Clusters were categorized as poultry-related based on laboratory and/or epidemiological data. Poultry-related *Salmonella* clusters were analyzed in STATA by serovar, cluster size, duration, and presence of poultry-related isolates; outbreaks related to poultry were also summarized.

Results: From May 2017-December 2023, 179/883 (20.3%) multi-jurisdictional *Salmonella* clusters were categorized as poultry-related, with Enteritidis being the most common serovar accounting for 100/179 (55.9%) of all poultry-related clusters. The majority (144/179; 80.4%) of clusters contained one or more poultry isolate (e.g., frozen breaded chicken, chicken animal, turkey food, turkey animal) from FoodNet Canada or the Canadian Integrated Program for Antimicrobial Resistance Surveillance sampling programs. There were 16 national outbreak investigations implicating frozen raw breaded chicken products as a likely source.

Significance: Advances in laboratory subtyping, alongside epidemiological investigations have identified a strong connection between upstream contamination in poultry products and environments, and downstream health impacts. Measures to reduce *Salmonella* in poultry products should be considered to reduce the overall burden of *Salmonella* in Canada.

T10-11 Retrospective Analysis of Historical *Listeria monocytogenes* Clinical and Non-Clinical Isolates from New York State between 2000 and 2021 Reveals Large Numbers of Small Localized Clusters

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Introduction: Listeriosis outbreaks may be difficult to identify because of the long incubation periods, and long-term persistence of *Listeria monocytogenes* in food production facilities leading to re-contamination of food products over a long period of time.

Purpose: The purpose of this study was to use whole genome sequencing (WGS) data to investigate historical *L. monocytogenes* isolates from New York state over the past 21 years (2000-2021) to identify sporadic and potentially outbreak-related cases, as well as their potential sources.

Methods: The genetic relatedness of the 517 clinical and 2,194 non-clinical isolates was obtained from NCBI Pathogen Detection. Clinical isolates were clustered based on Single Nucleotide Polymorphism (SNP) analysis with a threshold of ≤ 20 SNPs. Clusters were classified by time span, geographic location (i.e. county-level geolocation) and number of clinical isolates.

Results: In total, 323 clinical isolates were found to belong to 85 clusters with at least two isolates; 51 of these clusters included only 2 clinical isolates. Among all identified clusters, 15 had clinical isolates that were isolated 10 years apart. For clusters with 3 or more clinical isolates, clusters spanning more than 24 months were the most common (76 % of clusters). Approximately 56% and 66% of the 2- and 3-clinical isolate clusters contained cases that were not from contiguous counties. Several clinical isolates showed close genetic relatedness to food/animal/environmental isolates, suggesting possible sourc-

es of clinical cases could be identified.

Significance: Listeriosis outbreaks present some unique challenges to investigations when compared with other foodborne pathogens. *Listeria's* long-term persistence can lead to multiple cases temporally widespread linked to the same source. Identification of several closely related clinical isolates obtained more than 10 years apart suggests that several listeriosis outbreaks may be much longer than previously assumed, which demonstrates the significance of analyzing *L. monocytogenes* isolates over long-time frames in outbreak investigation.

T10-12 Thou Shall Not Pool: Low Certainty of Evidence and Substantial Heterogeneity Prevail in Systematic Review of Antimicrobial Drug Use in Cattle and Antimicrobial Resistance in *Salmonella* and Commensal *E. coli*

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Introduction: Predicting the public health impact of policies limiting antimicrobial use (AMU) in cattle requires quantifying the link between on-farm AMU and antimicrobial resistance (AMR) in foodborne pathogens.

Purpose: We conducted a systematic review and meta-analysis (SRMA) to evaluate AMR in enteric bacteria from cattle raised both conventionally (CONV) and without AMU (RWA).

Methods: We performed a SRMA using a predefined Population (Cattle), Intervention (AMU), Comparison (CONV vs. RWA), and Outcome (AMR in *Salmonella* or commensal *E. coli* from feces or beef) framework (PROSPERO Protocol #CRD42023399764). We evaluated evidence certainty using a GRADE approach, and between-study heterogeneity using the tau parameter. Associations between AMU and three definitions of AMR assessed at the isolate-level (tetracycline, ciprofloxacin, and third-generation cephalosporins (3GC)) were estimated with random effects Bayesian Binomial-Normal hierarchical models. Predictive intervals (PIs) incorporating heterogeneity were calculated with pooled effect sizes to illustrate the effect of between-study differences on association estimates.

Results: Out of 13,738 initial studies, 39 met the inclusion criteria, reporting results for 8,593 isolates. Evidence certainty ranged from low (tetracycline, 3GC) to very low (ciprofloxacin), indicating high potential for bias. Substantial heterogeneity was observed for all outcomes (tetracycline: tau=0.44, 95% Credible Interval: [0.16-0.76]; ciprofloxacin: 0.36[0-0.87]; 3GC: 0.39[0-0.95]. Low certainty of evidence and substantial heterogeneity suggest it is inappropriate to pool current studies describing AMU-AMR associations in enteric bacteria from cattle. PIs reflect non-significant associations for all AMR outcomes (tetracycline: odds ratio=1.73, 95% Credible Interval: [0.66,4.71]; ciprofloxacin:1.21[0.38,4.48]; 3GC:2.66[0.47,17.8]), in contrast with pooling alone (tetracycline:1.73[1.36,2.27]; ciprofloxacin:1.21[0.54,2.86]; 3GC:2.66[1.62,4.57]), illustrating the inappropriateness of pooling low certainty studies with substantial heterogeneity – as has been done in previous SRMAs.

Significance: Given the low certainty of evidence and heterogeneity, pooling studies to estimate AMU-AMR associations is not advisable. Divergence between pooled and predictive intervals further illustrate the impact of pooling biased studies with substantial heterogeneity.

T11-01 Aggregative Soil Sampling Shows Promising Indicator Organism Recovery in Comparison to Grab Soil Sampling from Commercial Romaine Fields

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Introduction: Aggregative boot cover sampling has the potential to be a more representative and powerful method for preharvest produce soil testing than grab sampling because it aggregates soil from more areas in a convenient way (walking vs scooping).

***Purpose*:** To develop (Year-1), optimize (Year-2), and use (Year-3) boot cover sampling as an alternative to composite soil grabs.

***Methods*:** Sampling was performed in commercial romaine lettuce fields in Salinas, California over 3 years. In Year-1, 28 dry boot covers, and 28 composite grabs were tested for generic *Escherichia coli*, total coliforms, and aerobic plate counts (APCs). 16S rRNA full-length sequencing was performed for selected samples. In Year-2, 38 hydrated boot covers, and 38 composite grabs were tested for the same indicators plus *E. coli* O157:H7 and *Salmonella* for selected samples. In Year-3, 40 hydrated boot covers, and 40 composite grabs were collected in response to large-scale flooding in the Salinas Valley and tested for generic *E. coli* and total coliforms.

***Results*:** In Year-1 developing stage, no generic *E. coli* was detected. Total coliform recovery from boot covers was 0.6 log CFU/g higher ($p<0.001$, Wilcoxon) than composite grabs. APCs were similar between boot covers (7.0 ± 0.3 log(CFU/g)) and composite grabs (7.1 ± 0.2 log CFU/g). Microbiome profiles were similar. In Year-2 optimization, we had similar trends on indicator organisms. No *E. coli* O157:H7 and *Salmonella* detected. In Year-3 responding to flooding, boot covers recovered 3.0 log CFU/g more ($p<0.001$) total coliforms than composite grabs, but no countable generic *E.coli* (LOD ranging from 0.3-1.1 log CFU/g) in boot covers verses 5 countable in composite grabs. Three years data of showed that generic *E. coli* was rarely detected, boot covers typically recover more total coliforms than composite grabs, and similar APCs recoveries.

Significance: These promising indicator organism recovery results justify further work to develop boot cover sampling in produce fields.

T11-02 Genomic Insights and Phenotypic Profiles of Novel Lactic Acid Bacteria Isolated from Artisanal Cheese for Use as Starter Cultures

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❖ *Developing Scientist Entrant*

Introduction: Lactic acid bacteria (LAB) are used in fermented dairy foods as starter cultures. LAB having typical starter culture traits and antimicrobial properties are ideal for creating safe and flavorful fermented dairy products.

***Purpose*:** Utilize genomics to mine for genes associated with desirable starter culture characteristics and antimicrobial production genes, then use culture-based techniques to confirm presence of these phenotypic traits.

Methods: Hundreds of LAB isolates were recovered from artisanal cheese; 39 of these underwent preliminary screening for antimicrobial production, and eight isolates' whole genomes were sequenced using Illumina MiSeq platform and were identified using the Genome Taxonomy Database. Mining for genes involved in proteolysis, sugar utilization and biogenic amine production was performed using NCBI tBlastn at 80% sequence coverage and identity cutoff. AntiSMASH software was used to reveal gene clusters involved in antimicrobial production. Culture-based assays were used to verify carbon utilization, antimicrobial production and casein hydrolysis capabilities.

***Results*:** Genomically, five out of the selected eight strains displayed a preference for galactose and lactose, while only three of these strains expressed the phenotype. When these three strains were grown using lactose as the sole carbon source, their populations were up to 3.6 times greater when compared to growth without a carbon source. Casein hydrolysis and flavor contribution genes (e.g., prtP) were found in three strains, and all hydrolyzed casein on skim milk agar. These three strains didn't contain genes for production of harmful biogenic amines such as tryptamine, putrescine and cadaverine. The three strains contained gene clusters for production of the antimicrobials enterocin X, lactococcin and polyketides. In a microtiter plate bioassay, the strains showed anti-Gram-positive activity, inhibiting *Pediococcus* growth by 1.6-to 4.1-times compared to the untreated control.

Significance: Three isolates exhibit potential for use in the fermented dairy industry to enhance product safety, sensory characteristics, and human health.

T11-03 Microbiological Risk Assessment of Biological Soil Amendments of Animal Origin and Corn Steep Liquor on the Attenuation of *Escherichia coli* in Organic Romaine Lettuce Production of California's Low Desert Region: 2022–2023 Season

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Introduction: Despite treatment, biological soil amendments of animal origin (BSAAO) may support the growth or survival of pathogenic *E. coli* in leafy green production. Additionally, there is a growing demand for non-BSAAO sugar-based organic liquid fertilizers (for example, corn-steep liquor) with unknown microbial food safety implications.

Purpose: Evaluate survival of inoculated rifampicin-resistant *E. coli* TVS 353 in soils amended with heat-treated poultry pellets (HTPP), seabird guano pellets (SBG), and corn steep liquor (CSL) used to grow organic romaine lettuce in California.

Methods: Eight BSAAO treatments including Control (no amendment), HTPP-1 (one application), and HTPP-2 two applications), SBG-1 and SBG-2, CSL, CSL+HTPP, and CSL+SGB in California's Imperial Valley during 2022-2023. Romaine lettuce transplants were planted in plots, and soils were inoculated with Rifampicin-resistant *E. coli* TVS353 (7-8 log CFU/mL) were applied to plots and were quantified via direct plating and most probable number (MPN) assays from soils and lettuce plants (at harvest date) over 42 days. Weather data and soil temperature and moisture data were recorded during the trial duration. Each treatment was performed in triplicate plots planted with romaine lettuce.

Results: All treatments showed a 3-5 log CFU/g reduction of *E. coli* in soils within 21 days and ~7 log CFU reduction by day 42 with no significant differences ($p>0.05$) between treatments. However, CSL treatments supported higher levels of *E. coli* on the lettuce plants compared with BSAAOs ($p<0.05$). Increased *E. coli* reduction in soils correlated positively with higher soil temperatures and longer sunlight exposures during the growing season.

Significance: The findings suggest that microbial risks associated with liquid-based fertilizer in combination with treated BSAAO may differ in organic lettuce production in the southwest desert.

T11-04 Prevalence of Shiga-Toxin Producing *Escherichia coli* and *Salmonella* in Pecan Orchards under Regenerative Agriculture Management

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❖ ***Developing Scientist Entrant***

Introduction: Adaptive multipaddock (AMP) grazing is an emerging regenerative agricultural practice which has shown to enhance soil health, improve animal and forage production, and simultaneously reduce soil greenhouse emissions. Currently, there is a strong interest in adapting AMP grazing in silvopasture of pecan production. However, it is unclear of the impact of AMP practice on food safety risks associated with grazing cattle in orchards.

Purpose: This study aims to evaluate the impact of AMP grazing on the prevalence of foodborne pathogens of Shiga toxin-producing *E. coli* (STEC), and *Salmonella* in pecan orchards.

Methods: Soil (April, July, and October) and in-shell pecan (December) samples were collected from three pecan orchards of Redriver pecan orchards, Love County, OK, under AMP management with a total of 43 samples per season. The detection and isolation of STEC and *Salmonella* were conducted through enrichment, selective isolation, and multiplex PCR. Grazing frequencies were recorded before collecting samples. Fisher's exact test and t-test analyzed pathogen detection rates.

Results: The prevalence of STEC (33.33%) and *Salmonella* (38.76%) in soil samples in 2023 (n=129) was significantly higher than in 2022 (n=129) (4.65 and 3.88%, respectively). Also, for the pecan samples similar patterns were observed where the detection of STEC (53.49%) and *Salmonella* (76.74%) in 2023 (n=39) was significantly higher than in 2022 (n=39) (15.38 and 15.38%, respectively). In both years, the detection rate of STEC in soil samples varied by season, with fall showing a significantly ($p<0.05$) higher rate than the other two seasons. The prevalence of *Salmonella* followed a similar trend as STEC, with the fall detection rates significantly higher than the other two seasons in both years.

Significance: The general observation is that the presence of the pathogens in the samples is more associated with season and recent grazing activity than with grazing frequency. However, such observation may change as the soil health continues to improve as the project progresses.

T11-05 Random Forest Models of Meteorological and Soil Health Effects on Presence of Generic *E. coli* in Fresh Produce Fields Grazed by Small Ruminants

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Introduction: Linear models predicting the presence of generic *E. coli* (gEc) in soil encounter limitations in meeting model assumptions due to the high proportion of gEc absences and the intricate interactions between meteorological and soil health factors. In this study, we collected soil and meteorological data from two field trials investigating natural fecal contamination through sheep/goat grazing in integrated crop-livestock systems (ICLS), and measured gEc as an indicator of fecal contamination and a surrogate of pathogenic Ec.

***Purpose*:** To identify and rank risk factors associated with the presence of gEc in ICLS using Random Forest (RF) analyses with incorporated machine learning.

Methods: Two separate RF analyses were performed with the outcome of gEc presence in the soil. For explanatory variables, the first model incorporated 80 meteorological variables including means/sums over different time intervals preceding soil sampling days with 1,032 sample profiles. The second model included 20 variables of soil characteristics with 257 sample profiles. Additionally, state (CA and MN), year, treatment (fallow, cover crop without grazing, and grazed by sheep/goats), sampling days, and soil moisture were added into each model. The importance of explanatory variables in models was evaluated by permutation feature importance scores.

***Results*:** The major influential factors associated with presence of gEc in soil included average soil temperature at 10 cm depth, maximum air temperature, wind direction for 3 days before soil sampling, cation exchange capacity and concentrations of manganese, sodium, and nitrate, along with treatment effects in each model. The predictive performances of the two models in the test sets were 71.0% and 76.9%, respectively.

Significance: By considering dynamic conditions of nature in the RF machine learning approach, potential environmental risk factors influencing the presence of gEc in an ICLS can be identified beyond treatment and sampling day effects, which can be generalized into different geographical regions.

T11-06 Risk Factors for Foodborne Pathogen Occurrence in the Production of Horticultural Food Crops in Ireland

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Introduction: Fruit and vegetables are at risk of microbiological contamination during production and processing from a range of sources including soil, irrigation water, wash water, processing equipment, staff etc. Washing cannot remove all contamination from fresh produce, so it is therefore imperative that all reasonable steps are taken to minimize microbial contamination during production, processing and transport. Growers need to know where risks may arise on farm so that targeted interventions can be implemented ensure produce safety by reducing the transference of pathogens onto crops.

***Purpose*:** The objective of this study was to undertake a mapping exercise to identify potential sources of contamination and the areas most likely to harbor foodborne pathogens in commercial horticultural settings in Ireland.

***Methods*:** Produce, water and environmental swab samples were collected from commercial strawberry, carrot and lettuce production units (n=9), on four different occasions. Each sample was tested for the presence of *Listeria monocytogenes, Salmonella* spp. and Shiga toxin producing *E. coli* by standard methodologies.

***Results*:** In total 620 samples were collected, including 473 environmental swabs, 38 produce samples and 109 water samples. None of the fresh produce samples were positive for the target pathogens. *L. monocytogenes* (n = 29 isolates) was detected in 13 samples (2.1%). Samples from six out of the nine sites harbored *L. monocytogenes* on at least one occasion. Isolates were mainly recovered from floor swabs, but also from conveyor belts, processing equipment and water. No *Salmonella* was detected in any sample. In total eight enrichments from the 620 samples tested positive for *stx* genes.

Significance: This study indicated that horticultural production settings can harbor pathogens, and in particular *L. monocytogenes,* particularly on surfaces, emphasizing the importance of rigorous cleaning and disinfection regimes and minimizing cross contamination of the crop.

T11-07 Survival of *Salmonella* and Generic *Escherichia coli* on Agricultural Ground Covers

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❖ ***Developing Scientist Entrant***

Introduction: Ground-covers are a horticultural practice implemented to improve plant growth and assist in pest management. Limited data exists on whether ground-covers used in produce production may pose a cross-contamination risk.

Purpose: This study evaluated survival of *Salmonella* on different ground-cover materials and generic *Escherichia coli* (g*EC*) on plastic mulch in different growing environments.

Methods: Biodegradable mulch, landscape fabric, and plastic mulch were constructed to fit 100x15mm plates. Coupons were spot-inoculated with a seven-strain rifampicin-resistant *Salmonella* cocktail and stored at 23°C, 55% RH (growth chamber). Only plastic mulch coupons were spot-inoculated with rifampicin-resistant green-fluorescent protein-tagged g*EC* and stored in a growth chamber, greenhouse (29°C, 50% RH), and field (24°C, 80% RH) environment. Coupons were enumerated for *Salmonella* and g*EC* at timepoints: 0, 0.06, 0.17, 1, 2, 3, 5, 7, 30, 60, 90, and 140d and 0, 0.06, 0.17, 0.41, 1, 2, 3, 5, and 7d, respectively. If counts were below the limit of detection, samples were enriched following modified FDA BAM methods. Summary statistics and significant differences in organism by time ($p \leq 0.05$) were determined by Tukey's HSD test in RStudio (V4.2.3).

Results: Biodegradable mulch had the lowest reduction (1.41±0.43 log CFU/cm^2) of *Salmonella* by 7d followed by landscape fabric (1.68±0.41 log CFU/cm^2) and plastic mulch (2.80±0.26 log CFU/cm^2). *Salmonella* survived >140d on all ground-cover materials. No significant differences were observed between biodegradable and plastic mulch reduction at 140d ($p > 0.05$). *E. coli* survived on growth chamber coupons for up to 7d (4.01±0.37 log CFU/cm^2) with a reduction of 1.86±0.40 log CFU/cm^2. By 7d, 2/30 greenhouse coupons had counts of g*EC*, while by 5d, g*EC* was negative upon enrichment in field coupons.

Significance: Ground-cover material impacted *Salmonella* survival and should be considered in risk management. *E. coli* survival was impacted by testing environment indicating not all production environments have the same risk.

T11-08 Understanding Climate-Sensitive Food Safety Risks of Pre-Harvest Foods in North America and Europe: A Scoping Review

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Introduction: Climate change is a real, present, and growing threat to food safety that will be further exacerbated by worsening climatic factors. However, the projected impacts of climate change on food safety are not fully understood.

Purpose: The objective of this research is to summarize the available literature regarding the impact of climatic factors on biological and chemical contaminants that could impact the food safety of preharvest foods in North America and Europe.

Methods: A scoping review was conducted to identify relevant academic and grey literature set in Canada, USA, and Europe. A comprehensive search strategy of keywords and MeSH terms of relevant biological and chemical contaminants, climatic factors, and preharvest foods was developed and applied in appropriate literature databases. Citations were deduplicated and screened by two independent reviewers against study inclusion criteria in two phases: one (1) title and abstract screening and two (2) full-text screening. Data from full-text articles were extracted and summarized according to identified climate factor-contaminant-preharvest food relationships.

Results: The initial search yielded a retrieval of 22,935 articles with 45 advancing to data extraction. Included articles were extracted and analyzed where climate factor-contaminant-preharvest food relationships identified included: (1) precipitation-*E. coli*-irrigation water, (2) temperature-*Salmonella* spp.-poultry, (3) temperature-*E. coli*-leafy green vegetables, (4) flooding-*E. coli*-irrigation water, (5) precipitation-mycotoxins-grains.

Significance: This research is novel in its synthesis of climate-sensitive biological and chemical contaminants that can harm the food safety of preharvest foods relevant to North America and Europe. This information will contribute to improved understanding of climate change impacts and potential adaptation strategies to increase climate resiliency and food safety at the preharvest level.

T12-01 Antibiotic Susceptibility Profiles and Pathogenic Potential of *Shewanella* spp. Isolated from Oysters and Seawater Collected from the Mid-Atlantic Region

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❖ ***Developing Scientist Entrant***

Introduction: *Shewanella* spp. include flesh-eating and food spoilage bacteria that can be transmitted through contaminated water and seafood. Immunocompromised individuals may be at increased risk of infection if raw or lightly cooked shellfish are consumed, or if cuts in the skin have been exposed to a contaminated marine environment. Many case studies on *Shewanella* infections resulted in death due to the lack of knowledge on appropriate antibiotics to use for treatment.

Purpose: The objective of this study was to evaluate 21 antibiotics that are commonly used to treat Gram-negative bacterial infections in humans and aquaculture products against six predominant *Shewanella* spp. recovered from oyster and water samples.

Methods: A total of 129 *Shewanella* isolates recovered from the Chesapeake and Maryland Coastal Bays, Maryland were tested against 21 antibiotics

using the Sensititre microbroth dilution method, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) and the FDA recommended standards. Tryptic soy agar with 5% sheep's blood was used to determine the hemolytic activity.

Results: Ninety-three percent of isolates were resistant to at least one antibiotic and 83% were multidrug resistant. Although 45% and 55% of isolates were alpha and beta hemolytic, respectively**,** there was no significant difference (p>0.05) in resistance between the two groups. However, there was a significant difference (p<0.05) in resistance between the different species. *S. algae* (97*%)* and *S. khirikhana* (96%) had the highest resistance profiles being resistant to 8 antibiotics, followed by *S. marisflavi* (37%) resistant to 6 antibiotics, *S. loihica* (47%) resistant to 4, *S. submarina* (100%) resistant to 3, and *S. seohaensis* (100%) resistant to 2 antibiotics. All species were susceptible to Levofloxacin.

Significance: This study provides insight on the antimicrobial resistance profiles of *Shewanella* against commonly used antibiotics. Frequent antibiotic resistance surveillance for *Shewanella* spp. is critical for understanding multidrug emergence among environmental isolates.

T12-02 Lipopeptide Biosurfactants as Potential Natural Preservatives against Food Spoilage Fungi

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Introduction: There is a growing demand to switch from chemical preservatives to natural, less toxic, and sustainable alternatives, which can extend the shelf-life of food products by inhibiting fungal growth.

Purpose: To evaluate the efficacy of lipopeptide biosurfactants against fungal spoilage strains and their interaction with several food ingredients.

Methods: Microdilution assays were performed in 96-well plates to determine the minimum inhibitory concentrations (MIC) of 6 lipopeptide compounds (A-F) against 5 filamentous fungi and 2 yeast strains. The compounds were screened in a concentration range of 0.5-64 µg/mL against *Aspergillus niger* D-02906, *Penicillium paneum* CBS 302.97, *Penicillium roqueforti* CBS 174.87, *Rhizopus stolonifer* CBS 819.97, *Eurotium rubrum* D-061178, *Hyphopichia burtonii* C-00349 and *Saccharomyces cerevisiae* NCYC 77. Calcium propionate was included as a chemical preservative reference. The interactive effect of the antifungal activity of compound B with different food ingredients (NaCl, oil, starch, and ascorbic acid) was investigated by adding these onto growth media at different concentrations to detect changes in the MIC.

Results: The MICs determined ranged from ≤0.5-8 µg/mL for lipopeptide B and within 1-16 µg/mL for compounds C, D and E. Starch and oil increased the MIC of compound B against *A. niger* and *P. paneum* by 2-8-fold, in concentrations as low as 1.25% (w/v) and 0.15% (v/v), respectively. Conversely, NaCl did not affect the MIC of this compound in concentrations up to 1.44% (w/v).

Significance: The results suggest that lipopeptides could potentially be used as natural preservatives in food products due to their strong antifungal activity. Additionally, understanding the interaction with different food ingredients gives better insights in which products these compounds could be used.

T12-03 Next-Generation Probiotics as Antibiotic Alternatives for Controlling *Campylobacter* Infections in the Food Chain

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Introduction: *Campylobacter jejuni* is a major cause of foodborne gastroenteritis worldwide. Poultry and poultry products are the main source of human infections. *Campylobacter* infections are normally treated using antibiotics, and there are no vaccines for protection against infections in humans or animals. The development of antimicrobial resistance has generated an urgency to develop alternative control strategies.

Objectives*:* We aim to evaluate the efficacy of next-generation probiotics (NGPs) on *Campylobacter in vitro.*

Methods*:* We evaluated the effect of 38 different probiotic strains on the growth of *C. jejuni* using an agar-well diffusion assay. The best probiotics were evaluated for their effect on *C. jejuni* growth when cocultured in broth media. The ability of probiotic supernatants to inhibit biofilm formation and preformed biofilm was evaluated. We also evaluated the effect of the selected probiotics on adhesion, invasion, and intracellular survival of *C. jejuni* in human intestinal cell lines. Additionally, the effect of probiotics on the expression of virulence-related genes was evaluated using RT-PCR. Each experiment was conducted at least two times.

Results*:* All the probiotics inhibited the growth of *C. jejuni*, however, we selected the top eight probiotics for further development. All the selected probiotics significantly inhibited *C. jejuni* growth when cocultured in broth media. Four out of eight probiotics demonstrated up to 100% inhibition of biofilm formation and preformed biofilms. All the selected probiotic strains significantly inhibited (p<0.05) adhesion, invasion, and intracellular survival of *C. jejuni* in human intestinal cells. Also, these probiotics significantly (p<0.05) decreased the expression of genes in *C. jejuni* that are involved in virulence factors, motility, invasion, and biofilm formation. Our future studies will focus on evaluating the effect of these probiotics on the colonization of *C. jejuni* in chickens.

Significance: Our results will facilitate the development of NGPs as antibiotic alternatives to control *Campylobacter* infections in food chain.

T12-04 Oral Supplementation of Trans Cinnamaldehyde Reduces Colonization of *Listeria monocytogenes* in Guinea Pigs and Downregulates Expression of Virulence Proteins

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is major foodborne pathogen that leads to life-threatening illness in humans. Due to ambiguous symptoms and emerging antibiotic resistance in *L. monocytogenes*, there is a need for developing novel strategies for controlling *L. monocytogenes* infections.

Purpose: This study investigated the effect of prophylactic supplementation of Trans cinnamaldehyde (TC; Generally Recognized as Safe status phytochemical from cinnamon) in reducing colonization of *L. monocytogenes* in guinea pigs.

Methods: For proteomics, LM ATCC-19115 strain was cultured either in the presence or absence (control) of sub-inhibitory concentration (SIC) of TC for 12 h followed by protein extraction and LC-MS/MS analysis. Differentially expressed proteins between control and treatment samples (n=3) were analyzed using Student's t-test (p<0.05). For guinea pig studies, 18 female Hartley guinea pigs were randomly divided in 3 groups (n=6/group; negative control, *L. monocytogenes* control, TC oil group). TC was administered daily by oral bolus at 32 mg/kg of body weight. All guinea pigs (except negative control) were challenged on day 14 with a 4-strain cocktail of *L. monocytogenes* (9 log CFU/animal). Necropsy was performed on day 28 followed by collection of small intestinal sections for enumeration of *L. monocytogenes*. Data were analyzed using chi square test at p<0.05.

Results: The SIC of TC down-regulated critical virulence proteins contributing to host cell invasion (InlA, InlB, InlH), intra-cellular spread (ActA), toxin production (Hly), protein synthesis (InfA), cell-to-cell spread (PlcA) and catalytic activity (PyrC, PyrF, PbpA, NagB) when compared to control (P<0.05). In control guinea pigs, 100% of jejunum, ileum and cecum samples and 55% of duodenum samples were positive for *L. monocytogenes*. Whereas, in guinea pigs supplemented with TC, a significant reduction of 35, 85, 65, and 55% of LM colonization was observed in duodenum, jejunum, ileum and cecum, respectively.

Significance: TC could be used as a natural prophylactic supplement to reduce colonization of *L. monocytogenes* in humans.

T12-05 Resistome Dynamics in Atlantic Salmon from Norway and Ireland

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Introduction: This study is part of CIRCLES (Controlling mIcRobiomes CircuLations for bEtter food Systems) which aims to discover and translate innovative microbiomes-tailored circular actions into concrete applications that will ultimately enhance EU food system performances and their overall sustainability. The setup of real-world labs in the field of 6 food systems relevant to the EU market – tomatoes, spinach, poultry, pigs, Atlantic salmon and seabream aquacultures – will enable CIRCLES to increase knowledge on the importance of food system microbiomes as determinants of productivity, quality, safety and sustainability.

Purpose: This work aims to characterize of antimicrobial resistance (AMR) in Atlantic salmon collected from Norway and Ireland.

Methods: More than 300 samples from salmon have been shotgun metagenomics sequenced. The samples were collected from different part of fish's body and different stage of life as well as from farmed, wild fish and environment. The shotgun metagenomic sequence data were analyzed to identify abundance, diversity and richness of resistome in Atlantic salmon's microbiome.

Results: AMR genes associated with β-lactams, tetracyclines, aminoglycosides, quinolones, sulfonamides, trimethoprim, phenicols, macrolides and fosfomycin resistance were found to be in high abundance in the salmon samples. Particularly, *lsa*(B), bla_{CME}, *msr*(D), bla_{OXA}, bla_{VEB}, *fosA*, *vga*(E) were among the most abundant AMR genes. The results showed that fertilized egg have different resistome compositions compared to the other developmental stages. Similar AMR gene abundance patterns were found among samples from aquaculture feed and fish developmental stages, suggesting that AMR genes could be from the environment and/or the feed which is provided to the salmon during their growth.

Significance: This work suggests that aquaculture feed could be a target source for reducing AMR in aquaculture.

T12-06 Sanitizer Solution Susceptibility of *Escherichia coli* O157:H7 Recovered from Inoculated Romaine Lettuce after Simulated Source or Forward Processing Conditions

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Introduction: Harvested lettuce is processed locally (source processing, SP) or at facilities remote to location of production (forward processing, FP). Recent *Escherichia coli* O157:H7 outbreaks with Romaine lettuce raised questions on possible acquisition of bacterial resistance to environmental stresses during processing.

Purpose*:* To assess *E. coli* O157:H7 susceptibility to sanitizer in rinse water after recovery from inoculated lettuce and following source or forward processing.

Methods*:* Leaves (n=3) from Romaine lettuce were spot-inoculated with ~7 log CFU/leaf *E. coli* O157:H7 EDL933 (ATCC 700927) or 2705C (2019 lettuce outbreak strain), bagged and source and forward processing simulated. Recovered cells were treated in solution with free chlorine (Cl), peroxyacetic acid (PAA), quaternary ammonium compounds (quats/QACs) or DI-water for 1 min before plating on MacConkey (MAC), with or without Tryptic Soy agar (TSA)-overlay. Injury was estimated by subtracting MAC counts from TSA-overlay counts. Quantitative-PCR after propidium monoazide treatment estimated viable-but-non-culturable (VBNC) cells by subtracting TSA-overlay counts from qPCR-estimated population. Data were analyzed with multiple regression.

Results*:* Processing condition, sanitizer and strain were significant factors for the survival of *E.coli* in rinse water (p<0.005). Overall, EDL933 counts (2.5 log CFU/g) were lower than 2705C (3.1 log CFU/g, p<0.001). While no difference in injury was found, VBNC level was higher in EDL933 (3.1 log) than 2705C (2.7 log, p<0.001). Survival on SP lettuce (2.9 log CFU/g) was higher than FP (2.6 log CFU/g, p<0.05). While no difference in injury level was detected between SP and FP, VBNC after FP (3.1 log) was higher than SP (2.8 log, p<0.01). Survival was higher in DI-water, 10 ppm PAA or 800 ppm QACs (>3 logCFU/g) when compared to 30 ppm PAA or 1 ppm Cl (<1 log CFU/g, p<0.001). QACs at 800 ppm caused less injury (-1.1 log, p<0.001) and VBNC (-1.3 log, p<0.001) than 10 ppm PAA.

Significance: *E. coli* O157:H7 recovered from Romaine lettuce increases sanitizer susceptibility with forward processing.

T12-07 Sodium Selenite Exhibits Antivirulence Effect on *Listeria monocytogenes* In Vitro

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is the third-leading cause of death among food-borne pathogens, causing severe gastrointestinal illness, septicemia, and abortion in humans. The key virulence factors, such as bacterial motility, hemolysin synthesis, and host tissue invasion contribute to *L. monocytogenes* pathogenesis. Inhibiting these factors could effectively control *L. monocytogenes* infection and positively enhance disease outcomes.

Purpose: This study investigated the efficacy of sodium selenite, an essential mineral in attenuating aforementioned *L. monocytogenes* virulence factors.

Methods: The sub-inhibitory concentration (SIC) and minimum inhibitory concentration (MIC) of sodium selenite against *L. monocytogenes* isolates Scott A and ATCC 19115 were determined. The isolates were cultured in Tryptic soy broth with and without SIC or MIC of sodium selenite, and *L. monocytogenes* motility, hemolysin production, and adhesion and invasion of human intestinal cells (Caco-2) were determined. Further, the effect of sodium selenite on major LM virulence genes was assessed using RT-qPCR. All experiments were replicated three times with duplicates, and data were analyzed using one-way ANOVA.

Results: The SIC and MIC of sodium selenite against *L. monocytogenes* were 0.26 µM and 462.59 µM, respectively. Sodium selenite at SIC and MIC significantly reduced all tested *L. monocytogenes* virulence factors (p < 0.05). At SIC and MIC, sodium selenite decreased *L. monocytogenes* motility by 41-47% and 57-67%, respectively, and hemolysin production by 100%. The adhesion and invasion of intestinal epithelial cells by *L. monocytogenes* were reduced by 70% and 78%, respectively in the presence of sodium selenite (p < 0.05). RT-qPCR data revealed that sodium selenite downregulated the transcription of major virulence genes, *inlA, InlB, plcA, plcB, motB* by at least two fold (p < 0.05).

Significance: Sodium selenite could potentially be used to control listeriosis; however, in-depth validation studies in appropriate animal models are necessary.

T12-08 Water in Cyclomethicone Emulsions are an Effective Non-Aqueous-Based Sanitizer against *Salmonella enterica* Enteritidis

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❖ ***Developing Scientist Entrant***

Introduction: Sanitation of low-moisture food (LMF) environments poses a unique challenge due the necessity of non-aqueous cleaning products. Currently, sanitation is often conducted by using alcohol-based cleaners; however, these compounds are highly flammable and require equipment to be

powered down. Our lab is innovating non-aqueous antimicrobials which have been demonstrated to be effective against desiccated *Salmonella*.

Purpose: Develop novel antimicrobial agents for sanitation of LMF processing environments using the non-polar solvent cyclomethicone.

Methods: *Salmonella* Enteritidis was desiccated at 75% relative humidity for 18-20 hours on stainless steel coupons. Treatment with cyclomethicone, acidified-cyclomethicone (0-500 mM acetic acid), and emulsified-cyclomethicone (stock emulsion: 10% span-80, 30% water, 60% ethanol; added in ratios from 1:9 to 1:49 to cyclomethicone) for 30-minutes at room temperature (25°C) was conducted. Microbial log reduction (MLR) was assessed using plate counts and calculated by $\log_{CFUnontreatment} - \log_{CFUtreatment}$. Significance ($p < 0.05$) was assessed using a one-way ANOVA with post-hoc Tukey's tests. Data are expressed as mean ± standard deviation.

Results: Three emulsions using the ratios 1:9, 1:14, and 1:29 significantly increased the MLR compared to cyclomethicone alone ($p < 0.01$, MLR = -0.12±0.46), cyclomethicone with 6% ethanol ($p < 0.001$, MLR = 0.83±1.48), and water in cyclomethicone emulsions ($p < 0.05$; MLR 1:29 = 0.63±0.50; MLR 1:49 = 0.39±0.16). Growth was below detectable limits (>5.5 MLR) for emulsions 1:9 (4/4 replicates), 1:14 (3/4), and 1:29 (3/4 replicates). No effect of acidification was identified (p value > 0.05) and the mean MLR was <1.5 for all tested formulations. However, variation was observed with some observations below detectable growth limits (250mM = 1/8 replicates; 500mM = 1/7 replicates).

Significance: Emulsified non-polar solvent based antimicrobials demonstrated promise for effective sanitation. These solvents could be especially beneficial for LMF processing environments and represent a novel method to clean food processing facilities with minimal water.

Poster Abstracts

P1-01 Food Safety Risk Perceptions – Foodborne Illness is Serious, But I'm Not Going to Get Sick

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Introduction: Food safety risk perceptions indicate how consumers perceive the risk associated with contracting foodborne illness which may influence how they handle and prepare food.

Purpose: To learn how U.S. consumers perceive the risk of contracting foodborne illness relative to COVID and the flu, and to better understand their risk perceptions for contracting foodborne illness relative to other events.

Methods: We conducted a national web-based survey of U.S. adults who cook raw meat and poultry (n=2,406).

Results: Although many respondents considered foodborne illness to be serious/very serious (84%) more so than COVID (74%) or the flu (72%), more than half thought it was somewhat/very unlikely they would get foodborne illness and about a third were not/not at all concerned about getting a foodborne illness. About 18% thought they were somewhat or very likely to contract a foodborne illness compared with 26% for COVID and 31% for flu. The level of concern was similar for the three illnesses: 44% said they were concerned/very concerned about getting a foodborne illness compared with 47% for COVID and 41% for flu. Respondents answered a ranking question to better understand their risk perceptions for contracting foodborne illness relative to five other events that could potentially occur during the next year. Most respondents were not very concerned about contracting foodborne illness relative to the other events—only 9% of respondents ranked it as their top concern, with nearly 50% ranking it fourth or fifth. Being in a car accident or contracting COVID was respondents' top concern based on the rankings.

Significance: The survey findings suggest that consumers understand that foodborne illness can be serious but do not consider it to be a personal risk. Food safety messaging needs to meaningfully convey the potential risk of illness if recommended food safety practices are not followed.

P1-02 A Publicly Available International Foodborne Outbreak Database, Why Would I Use That? Overview and Descriptive Analysis of Five Years of Requests

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Introduction: Since the year 2000, the Publicly Available International Foodborne Outbreak Database (PAIFOD) has been systematically collecting international foodborne outbreak data from publicly available sources to respond to stakeholder requests.

Purpose: To explore the range of stakeholders' needs and identify trends in requests.

Methods: Since March 2019 a log of requests including contacts, affiliated organizations, details of the request (pathogens of interest, food vehicles and countries, date ranges), and the purpose of requests has been recorded. Descriptive analysis was performed using Microsoft Excel 2016.

Results: Between March 2019 and December 2023 a total of 113 requests were made, peaking in 2019 with 32 requests. Requests were made by 18 different requesting organizations with the majority (98%, n=111/113) of organizations based in Canada. Other countries of requestors included the United States (n=1) and South Korea (n=1). Requesting organizations were primarily governmental (93%; n=70 Federal level, n=35 provincial level), followed by universities (6%, n=7/113). Most often requestors did not specify the type of pathogen (52%, n=59/113) but when it was, most requests looked for bacteria species (38%, n=43/113) in particular Salmonella (14% n=16/113). Similarly, most requests did not specify food items of interest (n=52). Seventeen requested did specify either pathogens or foods of interest but instead were interested in circumstances such as outbreaks involving caterers or festivals. The purpose of the request was identified in 94 requests (83%). By group type, most requests (33%, n=32/113) were for research projects including manuscripts, fact sheets, and student projects. The next most often were for risk assessment projects (23%, n=23/113) and policy (policy advice, briefs, advisory opinions - 17%, n=19/113).

Significance: PAIFOD has been a useful tool for researchers and governmental organizations since 2000 and while we have many stakeholders in Canada that consistently request reports from the database, there is little awareness and use outside of Canada.

P1-03 Consumers' Reflections on *Poisoned*: A Content Analysis of the YouTube Comments on the Documentary Trailer

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❖ ***Developing Scientist Entrant***

Introduction: Numerous methods have been used to deliver food safety information to consumers. The 2023 documentary Poisoned: The Dirty Truth About Your Food, which was available on Netflix and warned of toxins in commercially processed foods, drew significant attention. Little is known about the impact of this documentary on consumers' perceptions and trust in U.S. food systems.

Purpose: We conducted this study to evaluate the influence of food safety documentaries on consumers' attitudes toward food safety.

Methods: The trailer of the documentary *Poisoned: The Dirty Truth About Your Food* was posted on YouTube, from which we extracted the comments of 1,288 viewers on October 31, 2023, using a Google Chrome extension. We eliminated 96 (7%) viewer comments after screening with inclusion and exclusion criteria. We coded only English-language comments for general characteristics, emotion, and trust in food safety stakeholders. We assigned some comments more than one code to capture their full context.

Results: Our analysis encompassed 1,192 comments, most of which (n=900, 75%) discussed the topic of the documentary; only 19% (n=224) discussed the documentary itself. The most common theme articulated was skepticism about food supply safety (n=56, 4%). Among the comments, 41% expressed distrust in the U.S. food system, including distrust in the country (n=159, 12%) and distrust in the U.S. government (n=116, 9%). "Hopelessness" (n=63, 5%) and "fear" (n=58, 5%) were the emotions most commonly expressed among the 14% of comments articulating negative emotions toward the U.S. food system.

Significance: The documentary evoked negative emotions and distrust in the U.S. food system, which makes engaging consumers in food safety education more difficult to achieve. Further research is needed to better understand how food safety documentaries affect consumers' thoughts and behaviors.

P1-04 Cultivating Education: A Framework for Sustainable Mid-Day Meal Accessibility through Student-Maintained Rooftop Gardens in Primary Schools of Bangladesh

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✦ ***Undergraduate Student Award Entrant***

Introduction: Initiated in 2010, the Mid-Day Meal initiative in Bangladesh aimed to improve child nutrition, reduce primary school dropout rates, boost enrollment, and fight illiteracy. Despite positive reviews, barely 10% of schools currently implement the program due to funding and maintenance chal-

lenges, prompting the need for innovative solutions.

Purpose: This study aligns with the National Curriculum Framework 2021's emphasis on skill-based education by proposing a framework to improve Mid-Day Meal accessibility in primary schools through the implementation of student-maintained rooftop gardens.

Methods: The process includes having students maintain and instructors supervise the installation of prototype bedding systems on school roofs. Specifics include curriculum-integrated farming procedures, ongoing evaluations conducted during school weeks, and summative evaluations conducted twice a year to assess academic and agricultural advancement. To guarantee effective implementation, teachers, students, and community stakeholders are included in the joint endeavor. Quantitative data: Analyzing school enrollment, attendance, and dropout rates before and after ensuring mid-day meals, considering socio-economic factors. Qualitative data: Conducting semi-structured interviews with students, teachers, and parents to assess the impact on motivation, learning, and community engagement.

Results: Preliminary findings indicate that the proposed framework has the potential to improve Mid-Day Meal accessibility while fostering a generational movement for agriculture and farming in Bangladesh. The rooftop gardens provide a sustainable source of vegetables for the program, aligning with the Bangladesh National Curriculum Framework 2021's skill-based learning objectives. The study underscores the significance of active student engagement and community collaboration in achieving positive outcomes and nurturing a culture of self-sufficiency in food access management.

Significance: By resolving the difficulties associated with implementing the Mid-Day Meal program, this research represents a substantial contribution to food protection and public health. In Bangladesh, elementary education is approached holistically, and the inclusion of agriculture in the curriculum promotes both skill development and improved nutritional accessibility. The findings have broader implications for similar programs in other regions.

P1-05 Preliminary Efficacy and Acceptability of an Avatar-Based Mobile Phone App Promoting Food Safety Education in YBMSM Living with HIV

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Introduction: Food safety education has not been researched using a talking, teaching avatar in a mobile phone app for people living with HIV.

Purpose: Preliminary efficacy and acceptability of an educational, avatar-based food safety mobile phone app was determined among a high priority vulnerable population.

Methods: We recruited 254 young (18-34 years), Black men who have sex with men (YBMSM) living with HIV from 23 states and randomized 1:1 to a food safety app (n=129) or an adherence and retention in HIV care app (n=125). Food safety education was primarily provided audibly by female avatar. Nonoptimal health literacy was defined as correctly pronouncing fewer than 6 words from an adapted REALM-SF tool. Food safety knowledge was assessed before app download and after 6 months by scoring the number of correct responses to 9 questions on a 100-point scale. Mean scores pre- and post-intervention were analyzed using paired t-tests. Participants were asked if they would recommend the app and if the app affected their behavior in a positive way.

Results: 150 participants were retained and followed for 6 months, of which 38% had nonoptimal health literacy. On average for those with the food safety app (n=65), the mean knowledge score rose from 53.2% at baseline to 66.0% at 6 months (p<.001), whereas the score for the adherence group (n=85) did not significantly change (57.4% to 59.0%, p=0.49). Among those with the food safety app, 51/65 (78.5%) would recommend the app to someone else and 40/65 (61.5%) said that the app affected them in a positive way.

Significance: Our food safety mobile phone app shows promising preliminary efficacy and satisfactory participant acceptability in this sample of YBMSM living with HIV. These results suggest further development and refinement of this approach may be helpful in the field of food safety education, especially for vulnerable populations with low literacy.

P1-06 Evaluation of the Southern Center for FSMA Training and Lead Regional Coordination Center

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Introduction: Compliance with the Food Safety Modernization Act's Produce Safety Rule (PSR) and Preventive Controls for Human Foods Rule (PCHF) requires training, outreach, and technical assistance for owners and operators of small and medium-sized farms, beginning and socially disadvantaged farmers, small food processors, and small fruit and vegetable wholesale merchants.

Purpose: The Southern Center for FSMA Training is a consortium of 26 institutions aimed at enhancing produce safety in 14 southern states and 2 territories through a variety of educational means, including conducting and evaluating standardized courses (Produce Safety Alliance (PSA) and Food Safety Preventive Controls Alliance (FSPCA) Preventive Controls Qualified Individual (PCQI)) targeting the PSR and PCHF.

Methods: Validated pre/post-tests were given at PSA and PCQI courses to assess short-term knowledge gains from October 2022-January 2024 across the region. A qualitative evaluation was conducted with participants of both courses no less than four months after training to evaluate medium-term outcomes of behavior change related to food safety practices. Seventy-five PSA (1,230 participants) and 8 FSPCA (101 participants) courses were held across 11 and 1 states/territories, respectively.

Results: PSA post-test scores (20.35/25; n = 842) were significantly higher than pre-test scores (15.72/25; t = 14.33, p < 0.01), indicating a significant increase in knowledge. Of 13 practices included in the PSA surveys, the most adopted practices were writing or modifying farm food safety plans (58.08%;

79/136) and implementing new or different trainings for employees on food safety/hygiene protocols (57.55%; 80/139). Of seven practices included in the PCQI behavior change surveys (n = 24) the most frequent behavior change was that they recommended the training to others (n = 12), followed by fine-tuning existing food safety plans (n = 10) and implementing new food safety plans (n = 10).

Significance: Members of the SC have continued to create FSMA specific resources for growers and processors.

P1-07 An Evaluation of Food Safety Training Needs for Food Processors Through the Lens of Industry Professionals and Regulators in North Carolina

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Introduction: While food safety training is a regulatory and market-driven requirement, there is a lack of comprehensive and easily accessible training programs for frontline employees that address the needs of the manufacturing industry regarding top inspectional citations and concerns that are relevant to the industry.

Purpose: The purpose of this study was to assess the current needs and deficiencies of food safety practices among North Carolina food processors to inform the development of a comprehensive training program for food manufacturers through observations from industry professionals and state regulators.

Methods: Two separate Qualtrics surveys were emailed to over 200 industry professionals and North Carolina Department of Agriculture regulators. Both groups received the survey through an internal program listserv. The surveys consisted of likert scales, ranking, and short answer questions. Descriptive analyses using JMP were conducted to evaluate the results.

Results: Industry (n=63) reported personnel practices, such as employee hygiene and the improper use of personal protective equipment, as the greatest deficiencies in facilities (39%). However, regulators (n=14) reported a lack of adherence to standard operating procedures and improper documentation as the deficiencies most often observed during inspections (27%). Based on FDA's list of most common inspectional citations since 2018, both groups agreed that processing controls are the most critical factor in relation to safe manufacturing. However, the lack of employee training was ranked higher by regulators than by the industry. Based on short answers within the survey, regulators noted the need for a deeper understanding of food safety to support compliance of company policies.

Significance: This survey study identified the different deficiencies in food safety practices as seen by both regulators and industry professionals. Results showed the need for a training program that emphasizes the importance of comprehension of food safety for frontline workers to inform behavioral change.

P1-08 Transforming a Grower Needs Assessment into a Worker Training Toolkit

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Introduction: The Food Safety Modernization Act Produce Safety Rule establishes minimum standards for the safe growing, harvesting, handling, packing, and holding of produce, including worker training. A prior needs assessment was conducted and identified gaps in worker training competencies.

Purpose: The purpose of this work was to leverage needs assessment data into the development of a worker-informed farm training toolkit, consisting of: 1) an introductory video on risk assessment; 2) an interactive tool, worst-case farm scenario; 3) a visual, interactive tool, and printable SOPs; and 4) an interactive tool, this-or-that risk assessment through a harvesting day.

Methods: An interdisciplinary team met bi-weekly through 2023 to discuss the development, user experience, verbiage, and illustration choices for the four training resources. Various stakeholders helped to review and provide modifications throughout the development process.

Results: Based on the needs assessment (presented at Toronto), a toolkit that can be customized for a farm supervisor's worker training needs led to the following deliverables: a 30-second video introducing risk assessment and foodborne hazards; a visual and 7-step, interactive SOP highlights a worker preparing a bin for harvest and worker hygiene when working with animals; a worst-case farm scenario featuring14 discussion icons; and This-or-That error-based scenarios that take workers through the decision-making process they may experience during a day of harvesting or preparing a dunk tank using recirculated water. Three of the lessons provide "WHY" this behavior is important to public health, a best practice for adult education.

Significance: To our knowledge, there has not been a video to help raise awareness and educate workers on small to medium-sized produce farms on foodborne hazards in the context of risk assessment. Similarly, the development of printable and interactive worker training resources that are evidenced-based can increase farm operation buy-in and use of the toolkit to train and reinforce food-safe behaviors on the farm.

P1-09 Assessments of Need for Produce Safety Educational Resources for Non-English Speaking Produce Growers in the Midwest

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Introduction: There is a need to build educational capacity for small-scale refugee fresh produce growers. Social services organizations allow growers to create a new life with access to space to grow food and start their businesses. However, minimal food safety materials are available for these audiences in their native languages. Research reported that communicating with people who do not share the same first language is challenging.

Purpose: The project seeks to change the knowledge and mindset among small-scale refugee growers on food safety regulations through videos and poster resources.

Method: Before developing these resources, ten (10) refugee for-profit growers from Iowa and Kansas refugee growers of fruits and vegetables participated in focus group interviews. The participants were asked to answer questions about three main areas: workers' health and hygiene, biological soil amendment, and agricultural water.

Results: The focus group findings revealed that growers did not prioritize risks associated with Biological Soil Amendments (BSA), implementing best practices for their use, establishing protocols for sick workers, promoting grower wellness, testing water quality, and ensuring the maintenance of high wash water quality standards on the farms.

Significance: This research aims to safeguard public health, ensure the integrity of the food supply chain, and foster equity in agriculture. The team hopes to build institutional and regional capacity and foster collaboration among units that support refugee growers. These resources offer the refugee growers the information to comply with food safety regulations.

P1-10 Louisiana Food Retailer – Specialized Processing Methods eLearning Curriculum

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Introduction: The U.S. Food and Drug Administration identified specific processes to be conducted under strict operational procedures because of historical foodborne illness associated with these processes. In Louisiana, specialized processing and variance requirements are governed under the Louisiana Administrative Code Title 51(LAC 51) and enforced by Louisiana Department of Health (LDH). Needed assessment completed in Louisiana in 2020 showed that businesses under this regulation were "not aware" or "somewhat aware" of the food safety regulations, and level of training was reported from "no training" to "some training but not sufficient."

Purpose: This study aimed to develop a Specialized Processing Methods eLearning Curriculum targeting LDH Public Health Inspectors (PHI) and Louisiana Food Establishments and to evaluate the increase of knowledge of individuals completing the curriculum.

Methods: A nine-module Specialized Processing Methods eLearning curriculum was developed. Each module was independent from each other and contained presentation recordings, videos, presentations, and template files. The curriculum was launched through the LSU AgCenter Moodle. A pilot run was completed by LDH PHI. Participants completed a knowledge assessment before and after completing each module.

Results: A total of 104 participants completed the training. An increase of knowledge was observed across the completion of all modules. Pre-assessment knowledge was 75.43±19.92% compared to post-assessment with a 90.80±9.58%. The increase of knowledge for all the modules ranged from 8.60±11.09 % to 31.58±22.66%, "Module 2 -Food Safety Definitions" being the module with the least increase and "Module 1 -Where Do I Fit?" being the module with the greatest increase.

Significance: The implementation of this training curriculum will allow PHI and Louisiana food establishments to have access to education material to help them better understand regulatory requirements and increase food safety of products produced under a specialized processing method.

P1-11 Assessment of Food Safety Training Barriers for Underserved Value-Added Virginia Producers

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❖ ***Developing Scientist Entrant***

Introduction: Food manufacturing businesses, regardless of size, must comply with appropriate food safety education requirements, but guidance on effectively training small, minority producers is lacking in key areas, such as preferred formats and knowledge of current perceived barriers to compliance.

Purpose: To understand the greatest barriers to knowledge gain and behavior change for underserved value-added food producers in Virginia.

Methods: An anonymous 10-question survey was administered to Virginia food producers through links via email, flyers, electronic tablets, or hard copies in person. The survey queried demographic information about the producer, factors that inhibited their learning, and suggestions for improved education and outreach. Responses (n=43) were analyzed using descriptive statistics in R Studio version 4.2.3.

Results: Most respondents produced a single type of product (33/43), with fresh produce (15/43) and ready-to-eat products (15/43) being the most frequently reported. Most producers reported understanding that food safety can impact the success of their businesses (38/43). The most frequently reported situation that impacted producers' abilities to learn was an inconvenient training location (10/43), followed by the timing of the training (i.e., during work hours when trainings were preferred outside of regular work hours; 8/43). Preferred formats included factsheets (written information; 25/43), interactive (able to interact with the trainer and/or materials; 20/43), hybrid (17/43), prerecorded videos (13/43), presentations (13/43), one-on-one interactions (7/43), self-led trainings (7/43), and posters (3/43). Most (18/43) producers reported the primary reason food safety training was not effective was because they lacked the facilities to adequately implement food safety principles. Producers preferred to be contacted about food safety trainings by email (28/43) and flyers in the mail (11/43).

Significance: To reach parity in food safety education and outreach efforts, Extension educators should utilize a variety of teaching and outreach formats to meet the breadth of producer needs.

P1-12 Food Safety Culture Perception of the Brazilian Health Surveillance Inspectors

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Introduction: The food safety culture (FS-culture) is considered a risk factor for foodborne disease (FBD). The health inspectors may motivate the proactive evolvement of the FS-culture.

Purpose: To understand the perception of health surveillance inspectors regarding the FS-culture as a risk factor.

Methods: This is a qualitative study conducted with a sample of 24 health surveillance inspectors from ten cities in the central region of Rio Grande do Sul, Brazil. A focus group was used to conduct a storytelling session addressing elements of FS-culture as a risk factor for a case of a FBD outbreak (Shigellosis). The fictitious story was exclusively developed for the study. Subsequently, discussion among the inspectors was encouraged. The focus group lasted 90 minutes, was recorded, and transcribed. Thematic content analysis was employed to analyze the focus group transcription seeking to identify whether elements of FS-culture was considered in the discussion. The present study was approved by the Brazilian Ethics Committee (No. 5.828.021).

Results: The majority of the health surveillance inspectors were women (54%) with an average age of 43 years (Standard deviation: 9.6), and 71% had a university degree. The qualitative analysis resulted in the category "Causes and actors involved in the FDB outbreak", through the creation of subcategories: "Causes and perceptions of the FBD outbreak", "Leader's role", and "Food handler training". It was observed that the discussion was focused on food handling practices, indicating a limited perception regarding the FS-culture as a risk factor, despite two subcategories focusing on individuals.

Significance: A limited perception regarding the FS-culture as a risk factor was identified in the discussion of the health surveillance inspectors. The health inspectors can play an important role supporting food businesses to build a plan based on FS-culture.

P1-13 An Exploration of Doctoral Student Experiences of Recruiting Foodhandlers from Food Manufacturing and Food Service Sector Businesses for Food Safety Culture Research

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Introduction: In recent years, the awareness of food safety culture has grown significantly, emphasizing its crucial role in ensuring food business success. Despite a surge in food safety culture research studies, particularly by doctoral students, there remains a dearth of literature addressing the challenges encountered in recruiting participants from food businesses and engaging food handlers. While medical publications shed light on recruitment challenges in clinical trials, comparable insights for food safety culture research are notably scarce.

Purpose: The study aims to examine the challenges encountered by doctoral students in recruiting research participants from food manufacturing and food service businesses for food safety culture focused research.

Methods: A group discussion involving PhD and professional doctorate students (n=5) and doctoral research supervisors (n=2) was conducted to delve into their experiences.

Results: The findings reveal common challenges faced by doctoral students in recruitment, including reluctance from business owners/managers to

Poster

share project information with employees and fears of retribution among employees preventing participation. Successful recruitment strategies highlighted the importance of familiarity with the business, senior management endorsement, building rapport with food handlers, and offering incentives. However, online recruitment posed challenges due to fraudulent behavior aimed at obtaining offered incentives. Sector-specific nuances were discussed, with business owner recruitment proving more successful in the food service sector compared to food handler recruitment. Research supervisors emphasized the effectiveness of embedding doctoral students within businesses for successful projects. Reflecting on their experiences, the group discussion provided valuable recommendations for future food safety culture researchers.

Significance: This study contributes a comprehensive exploration of doctoral candidates' experiences in recruiting individuals and businesses for food safety culture research. While offering recommendations for future research approaches, it identifies the necessity for further investigations into the motivations and barriers for participation among food handlers. Such insights will guide future doctoral researchers in conducting successful food safety culture-focused research.

P1-14 Consumer Perspectives on Using Meal-Kit Boxes: A Mixed Methods Approach to Understanding Food Safety

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Introduction: Meal-kits provide fresh foods and pantry items with a recipe card, delivered directly to the consumer's home. Packaged in a cardboard box with cooling packs, recipe boxes are prepared ready for the consumer to use for domestic meal preparation and cooking. Limited UK research exists on the potential food safety risks associated with meal-kits and consumers awareness of these risks.

Purpose: The aim of this study was to establish consumers knowledge, attitudes and self-reported safe food handling practices associated with meal-kits through interviews and a questionnaire.

Methods: Using social media, volunteer and snowball sampling were used to recruit and interview meal-kit consumers (n=27) and a questionnaire distributed to the UK public (n=350). Qualitative analysis was performed using NVIVO. Descriptive frequency analysis was conducted using SPSS.

Results: Quantitative analysis found that 56% of meal-kit consumers always wash fruit and vegetables; but only 44% wash herbs: *"I've never- if I was buying stuff in the supermarket, I don't do it either. I should, I know, but I don't".* Consumers indicated 'always' handwashing before meal preparation (89%) while 78% 'always' wash hands after handling raw high-risk animal proteins: *"Yeah, so I've washed my hands at the beginning. And then after handling meat, those are probably the only- normally don't- more than that…".* Only 38% use a temperature probe during cooking while 89% agree that experience is a good indicator to judge when food is cooked: *"…if it was chicken let's say, I'd like cut into it to see…otherwise, I would just go by kind of go by eye and experience."*

Significance: Results indicate some food safety awareness among consumers and positive food safety practices, however, given that there is often a disconnect between self-reported practices and actual behavior future observational research could focus on consumers cooking a meal-kit in the domestic environment.

P1-15 A Study to Determine the Barriers of Operating an Effective Internal Audit System Within Wales's Food and Drink Manufacturing Sector

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Introduction: The Welsh Government has identified that to enable growth of the food and drink sector in Wales there is a need to support food and drink manufacturing businesses to proactively maintain food-safety scheme compliance. Internal audits (IA) are an essential tool for business to identify issues and focus on areas for continuous improvement. In businesses, implementation and application of IA can be inconsistent and reactive, leading to food safety nonconformities being raised in the businesses which could have been prevented.

Purpose: To determine the operational and implementation profile of IA systems in businesses in relation to their level of food safety compliance and level of proactivity.

Methods: A multiple-method approach included: desk-based research to determine Food Safety Scheme standard requirements, in-depth one-to-one interviews with food manufacturing businesses (n=10) of varying size and discipline, and a focus group with qualified scheme auditors (n=8).

Results: The study determined 80% of the businesses were certificated to a third-party food safety standard. However, only 40% of the businesses operated a multidisciplinary approach to the management of the IA system. Only 50% of the businesses IA team members held an externally recognized qualification. Concernedly, 20% of the businesses identified fewer non-conformances than external auditors. Only 40% of the IA team members were Root Cause Analysis trained which links to the lack of closure of corrective actions.

Significance: The operating profile of IA systems is variable. Its effectiveness is dependent upon multiple factors such as auditor independence, skills, capability, and resource. Findings have informed the development of support mechanisms to improve management commitment, food safety compliance and proactivity through the development and delivery of effective training and operational management. A series of bespoke workshops have been delivered to improve the effectiveness and impact of IA outcomes on food safety.

P1-16 Florida's Extension Programs Prepare Produce Growers for Produce Safety Rule Inspection

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Introduction: The Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR) was the first legislation to establish minimum standards for growing, harvesting, packing, and holding fresh produce.

Purpose: The new regulation meant Florida produce growers would require education and technical assistance to meet the requirements of the rule.

Methods: The University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) and the Florida Department of Agriculture and Consumer Services (FDACS) have collaborated, since 2017, to provide education and outreach through Produce Safety Alliance (PSA) Grower Training Courses and On-Farm Readiness Reviews (OFRR) to assist growers in meeting the requirements of the PSR. Pre- and post-tests are given at PSA Grower Trainings (n=1,559) to measure knowledge increase during the class. A follow up survey to determine practices changed or implemented is sent four months post training. Following the completion of each OFRR, surveys (n=72) are completed to evaluate farm readiness for rule implementation, and highlighted areas where more education was needed to meet minimum requirements.

Results: Post-test score means (21.66/25), were significantly higher than pre-test score means (18.05/25), indicating an increase in knowledge after participation in the training (t=-10.30, p< 0.05). Follow up surveys indicated the creation or modification of record keeping systems was the most common action taken based on the knowledge gained in the training. OFRR surveys indicated sanitation (36%), pre-harvest water (14%), and worker training (25%) required the most improvements, and that 46% of farms met the PSR requirements, 42% needed minor improvements, and 12% needed significant improvements to meet the FSMA PSR requirements.

Significance: The results demonstrated improvement of knowledge and practices of Florida farms regarding the FSMA PSR. As inspections continue throughout the state of Florida, education and outreach programs are ongoing to ensure Florida farms are prepared for implementation of the FSMA PSR.

P1-17 Exploring Knowledge, Attitudes, and Beliefs for Optimizing Food Safety Communication

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Introduction: Groups at higher risk for severe outcomes of foodborne illnesses include adults (65+ years), children (<5 years), immunocompromised people, and pregnant people. Social inequities have a role in foodborne illness risk as evidenced, in part, by higher rates of illness among people with lower incomes and Black/African American or Hispanic/Latino individuals.

Purpose: We sought to understand behaviors, knowledge, attitudes, and beliefs around foodborne illness and food safety among populations at risk for illness and severe outcomes.

Methods: We conducted 18 virtual focus groups, stratified by population of focus based on age, pregnancy status, child caregiver status, race/ethnicity and income level. We used grounded theory to analyze transcripts.

Results: The sample (n=113) included Black/African American (35%) and Hispanic/Latino (27%) participants; people who had lower incomes and/or lived in food deserts (40%); adults aged 65+ (21%); pregnant people (17%) and child caregivers (19%). Themes demonstrated participants' perceptions around foodborne illness risk, risk mitigation, and barriers to reducing risk. Older adults perceived their experience preparing food as a protective factor. Black/African American participants connected cleanliness to lowering risk of foodborne illness. Pregnant people expressed concerns about risk of food poisoning, with some concern about specific foods (e.g., deli meat, soft cheeses). Hispanic/Latino participants expressed concerns about risk, however many did not know about *Listeria*. Participants with lower incomes or who lived in food deserts discussed how "fresher" foods were lower risk but were seen as too expensive or inaccessible. Among caregivers, lack of time was seen as a barrier to engage in food safety practices.

Significance: The variability in knowledge and perceived risk shows the need to segment audiences when focusing communication or educational efforts to optimize food safety communication.

P1-18 Assessment of YouTube Videos for Vegetable Home Fermentation Misinformation

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Introduction: Successful home fermentation of low-acid vegetables relies on using correct amounts of salt, maintaining anaerobic conditions, and following basic sanitary standards for kitchen food preparation. YouTube (*http://www.youtube.com*) is a popular platform for presenting demonstration videos on topics that include home food fermentation. Yet, several studies have cast doubt on the credibility of YouTube as a source of health and food safety information.

Purpose: The aim of this study is to determine if information on home vegetable fermentation presented on YouTube is inconsistent with science-based best practices for fermentation and home food preparation food safety standards.

Methods: Ten vegetables were selected for evaluation. Searches were conducted using the stem word (ferment), the wild card symbol (*), and the vegetable name. Exclusion criteria were non-English and duplicate videos, pickling (addition of acid) videos, and those in which the vegetable was only a minor component of a mixture. The 10 most viewed videos for each vegetable were selected, and data were compiled for total view count, number of likes, time duration, and number of subscribers. Each of the videos was viewed and evaluated using a checklist created to record investigators' observations of incorrect fermentation practices and the extent to which violations of home food preparation sanitary practices occurred.

Results: One hundred videos with a combined viewership of 42.7 million were evaluated. The most popular vegetable types in descending order were cabbage, peppers, cucumbers, beets, tomatoes, carrots, garlic, onions, green beans, and cauliflower. The most often observed violations were failure to maintain anaerobic conditions, inaccurate salt measurement, lack of pH monitoring, and lapses in kitchen sanitation such as handwashing.

Significance: Currently, Extension recommendations are available for fermented cabbage and cucumbers. The results will be used for developing recommendations for home food preservers and in-service training for Extension educators.

P1-19 Evaluating Digital Accessibility of Ohio State University Extension's Home Food Preservation Fact Sheets

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Introduction: Extension fact sheets are commonly used to communicate safe food preservation methods to the public and may be housed on online platforms. It is essential to comply with web accessibility standards because individuals with impaired vision often rely on screen readers and other accessibility devices to assist their reading of information online. Fact sheets with errors or insufficient accessibility features could cause translation errors leading to unsafe food preservation practices.

Purpose: To evaluate Ohio State University Extension (OSUE) home food preservation fact sheets for compliance with the most recent Web Content Accessibility Guidelines (WCAG) 2.1.

Methods: OSUE fact sheets were accessed through the Ohio line platform and selected for inclusion in this study based on their home food preservation focus. The fact sheets were categorized by preservation method. Accessibility was evaluated using the WAVE ® (Web Accessibility Evaluation Tools) Browser Extension for Google Chrome, which evaluates websites for potential accessibility errors.

Results: Thirty-one fact sheets were evaluated and categorized as canning (15), freezing (7), drying (3), and other (6). All fact sheets were hosted in HTML format, which is recommended over PDF format for accessibility purposes. An average of 40 structural elements and 12 features, which improve accessibility, were identified across the fact sheets. An average of 2 errors, 1 contrast error, and 17 alerts (indicating potential errors), were identified.

Conclusion: Accessibility errors may lead to an inability of screen readers and other assistive devices to accurately relay important safety information for home preservation processes.

P1-20 Improving User Experiences (UX) with Food Safety Materials Developed by Agricultural Commodity Group: A Case Study of the Almond Industry

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❖ ***Developing Scientist Entrant***

Introduction: Agricultural commodity groups play a crucial role in developing and disseminating food safety materials among their stakeholders. Limited knowledge exists on how stakeholders use these materials and how to make them more compelling and more widely used.

Purpose: This project aims to examine food safety materials from the almond industry, identify insufficiencies, and suggest ways to enhance the experience of stakeholders using these materials.

Methods: This project comprises two studies. In Study 1, we conducted a content analysis of food safety materials found on the almond commodity group's website to understand material characteristics, such as topics covered, format, and target audience. In Study 2, we conducted semi-structured interviews with food safety managers from almond processors to gain insights into their material usage experiences and gather suggestions for improvement.

Results: Study 1 constituted an analysis of 46 food safety materials, the target audience for which included almond growers (70%), and processors (67%). Most materials (85%) focused on biological hazards controls. Largely consisting of manuals (26%) and fact sheets (22%), the materials emphasized

"what to do" but lacked experiential learning components that engaged the audience with hands-on activities. In Study 2, results from the interviews showed that processors had employee food safety training programs established. The materials from the commodity group were not the primary training source, but they were referenced during the development of training materials. Participants using these materials reported difficulty in (1) locating needed information, and (2) digesting materials that were too technical, thereby indicating the need to improve material accessibility and readability.

Significance: These findings deepen our understanding of stakeholders' needs and values, supporting the future development and improvement of food safety materials. User experiences (UX) should be considered when developing food safety materials.

P1-21 Project Expressing: A Qualitative, Quantitative, and Thermometry Study of the Hygiene Challenges Associated with Expressing Breastmilk in the Workplace

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Introduction: The benefits of breastfeeding are well-established, prompting many women to express breastmilk upon returning to work, thereby enabling infants to continue receiving breastmilk. However, along with this choice comes the added responsibility of maintaining hygiene for the equipment used and ensuring the safe storage of expressed breastmilk. The hygienic experiences of mothers expressing breastmilk in the workplace are relatively unexplored.

Purpose: One of the aims of "Project Expressing" was to explore the hygiene challenges associated with expressing breastmilk in the workplace.

Methods: The research involved three distinct phases: in-depth interviews (n=16) and online questionnaires (n=164) exploring the experiences of expressing in the workplace, followed by a thermometry study evaluating communal workplace refrigerators (n=25).

Results: Interviews revealed mothers expressed breastmilk at work to provide the *"best for baby"* for as long as possible, with 64% of questionnaire-respondents expressing to facilitate their return to work. Privacy and comfort were identified as crucial during interviews, but many mothers shared instances where suitable spaces for expressing and storing breastmilk were lacking. Nearly half of the questionnaire-respondents (48%) reported a lack of private space as a challenge for expressing. Concerns about the temperature of workplace refrigerators were expressed in interviews, and 35% of questionnaire respondents cited a lack of suitable workplace facilities for storing breastmilk as a challenge. Consequently, 30% reported not using workplace refrigerators, opting instead for cool bags/boxes with ice packs. The thermometry study identified widespread unsafe temperatures in communal workplace refrigerators, with mean temperatures exceeding the recommended 5.0°C in 86% of door storage areas and 77% of central storage areas. Furthermore, 96% of refrigerators lacked thermometers to monitor temperatures.

Significance: Sustaining breastfeeding by expressing in the workplace requires the mother's time and commitment, as well as a supportive workplace environment. This support includes a private space for expression and a safe environment to ensure hygienic expression and secure storage of breastmilk in the workplace.

P1-22 A Global Review of Foodborne Disease Incidence and Definitions Used by Food Safety Agencies to Determine Who are the Clinically Vulnerable Groups?

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Introduction: Food safety advice is especially important for groups within society who are at greater risk of severe illness, commonly referred to as clinically vulnerable groups. Food Standards Scotland commissioned independent researchers to review their current definition of "clinically vulnerable groups" to ensure appropriate food safety messaging.

Purpose: To evaluate the appropriateness of current definitions of clinically vulnerable groups in Scotland based on incidence data and global definitions.

Methods: Scottish and global data were analyzed to establish the incidence of *Campylobacter*, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes* and norovirus among the clinically vulnerable groups. Additionally, a review of clinically vulnerable definitions (n=87) utilized by global food safety agencies (n=30) was undertaken.

Results: The review of language used by global food safety agencies (n=30) to described vulnerable populations yielded 87 definitions. Overall, the definitions of vulnerability varied according to pathogens. Children were most frequently referred to as being vulnerable (n=26; 30%) in general definitions, and specifically *E. coli*, *Salmonella*, *Campylobacter,* norovirus. Pregnant women were mentioned in 21% of general definitions and 34% of definitions in relation to listeriosis. Similarly, the elderly, and neonates were frequently referred to in relation to listeriosis (30%, 14% respectively). Of peer-reviewed studies reporting foodborne disease incidence (n=2,805), 106 were identified for data extraction. Studies corroborated the language used by global agencies; however, they provided detail regarding specific conditions leading to vulnerability such as people with diabetes, or receiving cancer treatment were infrequently included in definitions despite increased incidence. Scottish incidence data suggested a significant increased risk of foodborne illness among people prescribed proton-pump inhibitors (34% campylobacteriosis, 25% salmonellosis, 20% listeriosis) while only one definition referred to this group.

Significance: Findings indicate adjustments to communication regarding clinically vulnerable groups are required and should be based on incidence data. Including reasons for elevated vulnerability may enhance consumer understanding.

P1-23 Development of Exciting Citizen Science Approaches to Investigate and Improve Home Food Safety Practices

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Introduction: Citizen science research involves the public collecting data. Currently, the approach is underutilized in a food safety context and warrants further exploration.

Purpose: Two citizen science initiatives were undertaken to equip the public with tools for ensuring food safety and to provide researchers with information on effective approaches for undertaking food safety citizen science projects.

Methods: Firstly, the "Is Your Fridge Cold Enough?" project distributed refrigerator thermometers (n=1,000) during summer 2023, whilst the Christmas Food Safety Citizen Science Project distributed cooking temperature probes (n=1,000) during December 2023. Online platforms were created to enable participants to enter temperature data including images.

Results: The "Is Your Fridge Cold Enough" project, involved in-person distribution at four festivals/events, with two distribution approaches; offering free thermometers with a chance to win £100 for reporting temperatures (12% response rate) and recruiting volunteers for the research without emphasizing the prize (21% response rate). The Christmas Food Safety Citizen Science Project relied on online advertising (calling for volunteers to participate in research with a chance to win £100) and postal distribution of probes. Within 12 hours of advertising 2,216 people had signed up to participate. The approach resulted in a 26% response rate. Motivation for participation included an interest in research involvement, protecting family from foodborne illnesses, and a desire for the free probe; financial incentive was not reported to be a primary driver.

Significance: This study provides insights into effective approaches for future consumer food safety citizen science projects. Contrary to expectations, online advertising and postal distribution proved efficient and effective, challenging the assumption that in-person distribution would yield a higher response rate. Data from both projects revealed food safety malpractices among consumers. However, the provision of information and tools enabled individuals to address issues like undercooking meat and unsafe refrigeration temperatures, promoting safer food in the home.

P1-24 Food Safety Practices among Consumers and Food Retailers in Three Districts of Buenos Aires City after COVID 19 Pandemic: Analysis of Consumer Interview Findings and Observations from Food Safety Experts

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Introduction: Proper food safety practices should be usual and systematic, however basic failures or lack of "food safety culture" are contributing factors to foodborne illnesses. Considering the recommended sanitary practices provided during COVID-19, we studied whether consumers and food retailers have incorporated food safety knowledge and habits resulting in a better food safety culture.

Purpose: Explore the level of food safety culture through the evaluation of knowledge and food safety behaviors in food retailers and their customers in 3 specific districts of Buenos Aires, Argentina.

Methods: 62 retail stores were observed by experts to assess hygiene and adherence to regulations, and 447 consumers were interviewed after leaving the stores.

Results: 1) Main motivation to choose the food retail store was proximity (53.9%), meanwhile cleanness was in the last place. 2) 60.4% of the people did not know the meaning of "cross-contamination". This percentage was higher in younger people and in consumers with lower educational level. 3) 20% of the people defrost food at room temperature. 4) 41% of consumers picked refrigerated/frozen food at the beginning of the purchase. 5) More than 50% said that they washed their hands for more than 30 seconds, and clean cans before storing them but most stopped using alcohol for hand disinfection.

Significance: The study showed that many consumers do not prioritize the hygiene of the stores when choosing them to buy food, that they are not aware of the cross-contamination concept, and that they don´t follow some of the most basic food safety practices. COVID-19 pandemic partially contributed to the creation of hygiene habits, so additional measures are needed to generate a genuine food safety culture among consumers. It is necessary to build food safety culture in the population by incorporating basic concepts into the educational programs from the primary school.

P1-25 Trends in Consumer Behavior Research: Key Findings to Improve Consumer Food Safety Practices

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Introduction: Several research studies have been conducted over the past five years that have explored consumer behaviors regarding food safety. This research includes observational meal preparation in test kitchens and provides valuable information for creating educational materials to improve consumer food safety.

Purpose: To present findings from recent consumer research regarding thermometer use and handwashing practices when cooking and handling different types of meat products and to use inform food safety educational efforts and messaging in order to reduce food borne illness from foods prepared at home.

Methods: Over the past five years, participants (n=747) were instructed to prepare a meal containing a meat product and ready-to-eat salad as they normally would at home while being videotaped. Coders viewed the videos 1) to determine the rate of thermometer use, 2) handwashing attempts, and 3) handwashing compliance associated with preparing and handling different types of meat products.

Results: Findings from this research show that thermometer use varies with the type of meat product being prepared (poultry, pork, and beef). Thermometer use was 34% for turkey burgers, 44% chicken thighs, and 77% for frozen stuffed chicken breasts. For breakfast sausage and bratwurst (pork), thermometer usage was 50% and 55% respectively. Participants used a thermometer to determine doneness of ground beef burgers 58% of the time. Handwashing also showed considerable variation depending on the type of meat product. The handwashing rates for chicken thighs and sausages/bratwurst/burgers were similar (17-26%), whereas the rate for the frozen, not-ready-to-eat breaded chicken was 3%.

Significance: These results highlight the potential to create more targeted messages to consumers to address the observed differences in behavior when handling and preparing specific types of meats.

P1-26 Exploring Illnesses Reporting Practices among Foodservice Employees

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Introduction: The data from CDC showed that more than 40% of the foodborne illness outbreaks were caused by food handled by foodservice employees who were ill.

Purpose: To understand the phenomenon of foodservice employees coming to work when they were ill and to investigate challenges in reporting illnesses among this group of individuals.

Methods: Thirty participants were recruited through Prolific, a market research company. They were asked a series of open-ended questions related to the key factors affecting their decisions to come to work while feeling unwell, the strategies they employed to reduce the risk of contamination while working with food, and the food safety policy (e.g., sickness reporting system and protocol) of their foodservice establishments. Their responses were independently coded by two researchers, and themes were identified.

Results: All participants have gone to work despite feeling ill in the past one year. They came to work due to personal factors, such as financial situation and personal attitudes (i.e., additional work for coworkers, commitment to work). Additionally, work-related factors, including job security and opportunity for promotion and treatment at work (retaliation, mistreatment at job) also affected their decisions to come to work. About 40% of the participants (n=12) indicated they did not handle food while sick; If they handled food, they wore gloves and masks, washed hands more frequently, and/or used hand sanitizers. Twenty participants felt that their workplace was accommodative when they were ill. The management should set an example, establish a positive workplace environment (i.e., trust, supportive, care, respect, clear communication), and provide benefits (paid time-off and insurance) to their employees to encourage them to report their sickness.

Significance: The study reveals that importance of supportive workplace environments in sickness reporting in the foodservice setting and provides insights for the policymakers to develop strategies that prioritize consumer health without compromising employees' well-being.

P1-27 A Qualitative Study on Tools to Practice Food Safety on Farm to Minority Farmers: A Case Study of Hmong Farmers in Missouri

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Introduction: While previous studies have indicated the need for developing hands-on practices using food safety tools for Hmong farmers, the current Food Safety Modernization Act (FSMA) training program does not address these specific needs.

Purpose: This study aimed to investigate the specific needs and barriers related to the use of tools for practicing food safety among minority farmers, using a qualitative approach.

Methods: Six Hmong farmers and six Produce Safety Alliance (PSA) trainers with prior Hmong farmer experience participated. Each farmer participated in a 60-minute interview to identify their specific needs and barriers regarding the use of tools for practicing food safety, with a fluent Hmong-English translator's assistance. Additionally, each PSA trainer underwent a 60-minute interview to discuss their experiences and challenges in training Hmong

farmers on the same topic. All interviews were recorded, transcribed verbatim, and used to initially identify key concepts and themes. Observations of food safety handling behaviors were conducted at six Hmong farms.

Results: Hmong farmers mentioned the need for specific tools to facilitate food safety practices on their farms, including washing stations equipped with plumbing and hoses, storage containers (e.g., buckets, barrels, and totes), dedicated storage rooms, coolers, essential chemicals (e.g., test strips), and various covering structures (e.g., shelters, plastic sheets, and ground covers). On the other hand, PSA trainers emphasized the importance of written documentation and tools such as record-keeping templates, clipboards, and waterproof notebooks to support effective food safety practices. Both farmers and PSA trainers identified cost as a significant barrier that hinders Hmong farmers from adopting FSMA practices, and they both acknowledged the challenges associated with record-keeping.

Significance: Through an exploration of the needs and barriers associated with the use of tools for practicing food safety among minority farmers, this study aims to make a meaningful contribution by extending support and empowerment to underserved communities.

P1-28 A Pilot Survey of Athens–Clarke County, Georgia Community Food Environments and Food-Related Behaviors

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Introduction: Consumer food procurement behaviors and motivations whilst navigating their respective food environments influence food hazard exposure and diet quality.

Purpose: Capture how Athens-Clarke County, GA (Athens) residents perceive and navigate their food environments.

Methods: A pilot Qualtrics survey inquired into food safety behaviors, food security status, and food procurement practices, perceptions, and motivations of Athens residents. The survey was distributed through snowball sampling through social media groups and listservs associated with the University of Georgia (UGA) Cooperative Extension and members of the research team. Data were collected under UGA IRB ID PROJECT00008612.

Results: The survey received 116 responses. Most respondents procured food from grocery stores (100%) and farmers' markets (34%), with 78% indicating they tended to shop at the retailer(s) closest to their home. Residents who did not typically shop at the retailer(s) closest to home indicated produce quality (27%), food option variety (25%), food pricing (23%), and customer service quality (13%) as primary motivations driving this behavior. Seventy percent perceived fresh produce quality to differ between different retailers and/or areas of town, citing differences in variety, quality, shelf life, cleanliness, and presentation of fresh produce. When selecting which fresh produce item to buy, respondents ranked signs of physical damage or of mold or rot as the most important factors they consider. Approximately 93% of respondents often or always rinsed their produce before consumption, and 92% of respondents sourced their water from the municipal water supply in Athens. Seventy-six percent of respondents always washed their hands prior to preparing food; 23% reported doing so often, but not always. Fourteen percent of respondents experienced a household member becoming ill with suspected foodborne illness in the past year.

Significance: Ongoing efforts to better understand consumer experiences and practices navigating their food environments are foundational to improving consumer food safety guidance.

P1-29 Food Safety Recommendation for Traditional Fermented Food for Small-Scale Producers: Injera as a Model

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Introduction: Injera is a traditional fermented food originally from Ethiopia, that is becoming popular in western countries. It is a fermented pancake-like bread prepared primarily with teff flour but other cereals like wheat, barley, sorghum, maize flour can be used. Injera production involves two rounds of spontaneous fermentations lasting 1 to 7 days. There are no records of foodborne outbreaks associated with injera in the United States. This may be due low percentage of individuals going to the doctor after illness or weakness in the outbreak surveillance system. Nevertheless, foodborne pathogens may be introduced during the production of injera.

Purpose: The goal of this study is to review the production on injera and create a food safety guidance for small-scale producer.

Methods: We searched and reviewed available peer reviewed publications, blogs and online videos and summarized different injera production. We created a flow chart and identified critical control points, critical measures, and control measures for the safe production of injera during fermentation and storage.

Results: The pH of the batter at the initial fermentation must drop to 4.40 or below after 48 hours. If not, product should be discarded. Contamination can occur from low quality raw ingredients inadequate hygiene in the manufacturing facility, improper storage conditions. The starter culture (ersho) should be handled with care to prevent cross-contamination with pathogens or other spoilage organisms. Finally, injera should be cooked at least 165°F for at least 15 seconds. It is the producer's responsibility to ensure the ingredients, preparations and storage conditions meet food safety within their respective jurisdiction.

Significance: There is an urgent need for food safety standard specifically for small scale producers as food systems in the West is rapidly diversifying. The information provides guidance pertinent safety recommendation for the production injera focusing on small-scale production.

P1-30 A Multiplex Digital PCR Method for Screening All EU-Authorized GMO Events for Food and Feed

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Introduction: The use of genetically modified organisms (GMOs) has caused debate in human and environmental safety, labelling and consumer informed choice. To be commercialized within the EU, the GMO event must be approved and any product containing over 0.9% GMO must be labelled. The use of protein-based methods, gold-standard for GMO screening, is hindered by the high processing levels of food/feed matrices. Among DNA-based methods, digital PCR (dPCR) allows absolute quantification, higher multiplexing capabilities, reduced inhibition and decreased quantification uncertainty.

Purpose: The goal of this study was to develop a dPCR-based method for screening 100% of EU authorized GMO events for food/feed, according to EU Joint Research Centre (JRC) guidelines.

Methods: The primers/probe sets used were retrieved from the JRC and organized in 5 panels. These panels target the most common elements (P-FMV, P-35S, T-*nos*, *pat* and *cry* - covering 96% of EU authorized events), the remaining events (MON87769, DP-305423-1, BPS-CV127-9, MON87708, DAS-40278-9 and DP-073496-4) and the respective taxon targets. The method was optimized and validated using certified reference material (CRM) in different quantification levels (100%, 10%, 0.1% and 1%) and following ISO 20395:2019 guidelines, which included sensitivity, specificity, robustness, linearity and uncertainty assessment.

Results: All panels were adequate for relative quantification as the DNA quantification matched the weight/weight ratio of the CRM with a CV lower than 35%. The method's dynamic range is between 100% and 0.1% GMO quantification, the latter being the method's Limit of Quantification. All the parameters for uncertainty measurement were within JRC's guidelines. Method was successfully applied to different food/feed samples.

Significance: This ready-to-use dPCR kit allows a fast and reliable screening of 100% EU authorized GMO events in food/feed, with a quantification limit of 0.1% GMO content. Among all tested samples, 37.5% were positive ([0.18%; 41.16%] GMO event) and only 12.5% reached higher quantification than EU's labelling threshold, necessary for sample import ([3.38%; 41.16%] GMO event).

P1-31 Exposure of Soybean to Wildfire Nanoparticles (WFPMs) Leads to High Levels of PAHs in Soybean

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Introduction: Wildfire events are becoming frequent across the United States. Wildfire-generated smoke is composed of particulate pollutants consisting approximately of 90% of fine particles (≤ 2.5 µm of diameter) named wildfire nanoparticles (WFPMs). These particulates can deposit onto agricultural crops, resulting in an uncharacterized exposure pathway with unknown effects and implications on both the plant and humans or animals that consume the plant.

Purpose: Taking the example of soybean, the present study evaluated the levels of PAHs in soybean plants after exposure to synthetized wildfire nanoparticles.

Methods: To expose soybean plants to WFPMs, soybean plants were dipped in 100 and 500 µg/mL concentrations of in-house synthetized WFPMs. Subsequently, two weeks after exposure, plants were harvested, biomass data collected and immediately frozen at -20ºC prior to further determination of PAHs contents. Levels of PAHs in WFPS and plant material were assessed using a validated GC-MS method. Extraction of PAHs from WFPS was achieved by maceration of 1 mg of the material for 18h in acetonitrile, filtration with ACRODISC® nylon filters 0.45 µm into vials for GC-MS analysis. For soybean leaves, starting with 3g of fresh leaves, a QUECHERS method was used to extract PAHs by using acetonitrile as extractant and a mixture of sulfate magnesium and ethyl acetate sodium salts as aqueous phase separators.

Results: The 16 EPA priority PAHs were detected in WFPMs and exposed soybean, at various levels with ∑ PAHs ranging from 746.35 to 7619.94 ppb. Those levels are higher than the U.S. Environmental Water Protection Agency recommended, 1 to 4 ppb for individual PAHs.

Significance: Considering that a number of the detected PAHs are documented to be carcinogenic and have been linked to cardiovascular diseases and poor fetal development, crops exposure to WFPMs could become an issue of great concern for consumer health.

P1-32 Review of Three Years of Comprehensive Active Surveillance Program (CASPr) in Detection and Identification of Foodborne Pathogens

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Introduction: The targeted focus of outbreak commodity contributed to an increase of positive pathogens in Public Health Command, West.

Purpose: The objective of this report is to look at the effectiveness of the Defense Public Health Comprehensive Active Surveillance Program (CASPr) from its initiation in 2019 to 2022. CASPr targets one certain commodity and curates the remainder food monitoring program for each region.

Methods: I conducted a search of the Public Health Command electronic Laboratory Information Management System (eLIMS) for the Food Analysis and Diagnostic Laboratory. The search parameters were all samples between 2019 and 2022. The samples were analyzed for 'Out of Specification' (OOS), a label in the system that denotes a positive sample. These OOS samples were analyzed according to commodity and pathogen. The results were cross analyzed with the CASPr schedules.

Results: After analyzing 24,924 samples that included over 100,000 tests and 2,506 OOS samples and comparing 2019 samples to each year after the introduction of CASPr, 2019 and 2020 had a 14.79% higher OOS rate (p<0.01). 2019 and 2021 had a 49.43% higher OOS rate (p<0.01). 2019 and 2022 had a 0.16% lower OOS rate (p>0.01), which was not significant and cannot be dismissed due to CASPr schedule. Further analysis showed OOS increased more than 50% in targeted unapproved commodities that include hummus, cheese, and Ready-to-eat items. Confirmed pathogens detected in the OOS samples were *Listeria spp*., *C. perfringens*, *Salmonella spp*., and *B. cereus*.

Significance: The data suggests that targeting unapproved sources and curating the quarterly CASPr schedule by commodity and regional outbreaks helps to detect and identify foodborne pathogens and could prevent military outbreaks.

P1-33 Development of a PCR-Based Lateral Flow Immunoassay for the Identification of Rainbow Trout Ingredient in Foods

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Introduction: Rainbow trout (*Oncorhyncus mykiss*) is a freshwater-farmed fish and is often used as the adulterant of Atlantic salmon by some merchants as it is considerably less expensive but with similar physical appearance. However, rainbow trout may exhibit a higher prevalence of parasites (***e.g.***, rumen fluke, intestinal hookworm, etc.) and diseases, therefore causing potential food safety concerns.

Purpose: A simple polymerase chain reaction (PCR)-based lateral flow immunoassay (LFIA) was developed in this study to identify rainbow trout ingredient in foods.

Methods: This assay was developed by coupling PCR amplification of rainbow trout DNA with the detection of labeled amplicon using LFIA.

Results: The rainbow trout-specific PCR primers targeted at mitochondrial ATP6 gene were successfully designed. The color signals of LFIA could be observed by the naked eye within 5 min. The reliability of this assay was confirmed by quantitative real-time PCR (qPCR). The proposed assay could achieve an LOD at 0.1 ng/µL of rainbow trout DNA. The specificity of the primers was validated against 17 other species related to fish meat fraud and no cross-reactivity was identified, followed by successful test of commercial products for fish meat authentication.

Significance: PCR-LFIA is simpler and faster than the conventional qPCR and has great potential to be applied to rapidly authenticate rainbow trout ingredient in foods.

P1-34 Use of DNA Barcoding to Detect Adulteration in Ginseng Supplements

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❖ ***Developing Scientist Entrant***

Introduction: The pandemic led to significant increases in demand for dietary supplements, including ginseng supplements, to purportedly support immune health and provide other health benefits; however, this heightened demand has subsequently increased the risk of adulteration in these supplements.

Purpose: The objective of this study was to use DNA barcoding to identify species in ginseng dietary supplements.

Methods: A total of 50 dietary supplement products containing ginseng (*Panax ginseng, Panax quinquefolius,* or *Panax notoginseng*) were tested with the following DNA barcoding regions: ITS2, *rbcL*, and *matK*. Two composite samples were tested per supplement. The consensus sequences were queried against GenBank to identify the species in each sample. RStudio was the statistical software used to compare sequencing success and amplification success across the genetic markers for species identification.

Results: Of the 50 products tested and confirmed via DNA barcoding, at least one species was identified in 68% of products (n=34), and the expected species was identified in 52% of products (n=26). Undeclared plant species, such as rice (*Oryza* spp.) and avocado (*Persea americana*), were detected in 11 products. The presence of undeclared plant species may be due to factors such as cross-contamination during harvest or processing, or intentional substitution for economic gain. Of the three genetic barcoding loci used, *matK* presented the greatest sequencing success when testing samples, with a sequencing rate of 60% (n=31).

Significance: Overall, these results reveal the challenges of using DNA-based methods to authenticate ginseng dietary supplements. The combination of

DNA barcoding with a targeted approach may improve the species identification rates in ginseng dietary supplements.

P1-35 Using Foodomics for Food Authentication: The Case of Grass-Finished Beef

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Introduction: Producers spend time and resources grass-finishing their animals using regenerative agriculture practices, and consumers are willing to pay premiums to purchase grass-finished beef.

Purpose: Using a multi-omics approach, the goal was to measure fatty acids and secondary metabolites in beef from different dietary groups to identify metabolic pathways and genes of interest to build a robust grass-finished beef authentication database.

Methods: This two-year study (2020-2021) involved 115 steers that were randomly allocated to one of four diets: 1) pastured with hay (control), 2) pastured with baleage, 3) pastured with soybean hull, or 4) baleage with soybean hulls in confinement. Fatty acids were measured using GC-MS, while secondary metabolites and vitamins were measured using UPLC-MS/MS. Statistical visualization of metabolomics data was performed using MetaboAnalyst for supervised Random Forest classification and the identification of metabolic pathways. The KEGG library for *Bos taurus* with a hypergeometric test was used, while topology analysis was conducted using the relative-betweenness centrality method. Target genes were identified based on measured metabolites and most impacted pathways, followed by a literature review on PubMed and Google Scholar using the keywords "gene expression," "fatty acids," "secondary metabolites," and "animal health."

Results: Vitamin E, the n-6:n-3 ratio, lipid oxidation values, and long-chain n-3 fatty acids were the top discriminating compounds. Beef samples were separated by dietary group with a degree of certainty (73%). The metabolic pathway analysis revealed that the most impacted pathways were related to the biosynthesis of unsaturated fatty acids, citrate cycle, purine, arginine, glyoxylate, and dicarboxylate metabolism ($p<0.05$). Based on these results and the literature search, PPARγ, FABP5, EIF4EBP1, NPNT, SELENOW were identified as potential target genes.

Significance: Authentication of grass-finished beef using metabolomics and transcriptomics would be of economic and health benefit as it would increase producers' revenues and consumer confidence in products of grassland origins.

P1-36 Quality Comparison in Phenol Content and Antioxidant Activity of Different Oolong Teas: Application of Chemometrics

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❖ ***Developing Scientist Entrant***

Introduction: Taiwan oolong tea is a popular non-alcoholic beverage consumed by the population. However, due to its low production rate, inferior-quality oolong tea from Vietnam, China, and Indonesia is imported into Taiwan, compromising the superior Taiwan oolong tea quality.

Purpose: To perform quality evaluations of different oolong teas by phenolic content, flavonoid content, and antioxidant activity. Further, chemometric methods were employed for the classification purpose.

Methods: Four oolong tea samples were collected from four different origins (Taiwan, Vietnam, China, and Indonesia). Teas were extracted with 80:20 methanol: water and using a 1:50 sample-to-solvent ratio. The total phenolic content (TPC) and total flavonoid content (TFC) were investigated. The antioxidant activity was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Three chemometric methods, namely, principal component analysis (PCA), canonical discriminant analysis (CDA), and hierarchical cluster analysis (HCA), were employed for the classification of oolong tea samples.

Results: The highest TPC was found in oolong tea extracts from Taiwan (18.12 ± 0.12 mg/g), followed by Vietnam (14.06 ± 0.07 mg/g), China (11.11 ± 0.12 mg/g), and Indonesia (10.05 ± 0.10 mg/g). On the other hand, the highest TFC was presented by oolong tea extracts from Indonesia (5.30 ± 0.26 mg/g), while the lowest was from China (4.35 ± 0.03 mg/g). The % DPPH inhibition ranged from 72.20 ± 0.84% (Vietnam) to 74.83 ± 0.42% (China). All three chemometric methods classified oolong tea samples into four groups. The PCA contributed to the cumulative classification rate of 88.209% (PC1 = 47.607% and PC2 = 40.602%). CDA exhibited a correct classification rate of 100% for the first three canonical discriminant functions. Additionally, the HCA dendrogram showed the separation of four classes.

Significance: Chemometric methods could be applied in the classification of oolong tea based on total phenol and antioxidant activity for quality and control purposes.

P1-37 The Role of Microbiological Criteria Worldwide in Controlling the Possible Incoming Outbreaks of Shiga-Toxin Producing *Escherichia coli* (STEC) Notably the Strain O157 from Food Matrix

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Introduction: STEC causes hemolytic uremic syndrome (HUS) in humans and normally is associated with consumed food, particularly undercooked meat. Many countries establish microbiological criteria to control foodborne pathogens in food matrices, especially the most well-known strain of STEC, *E. coli* O157. However, some other countries do not include *E. coli* O157 and other important pathogens in their microbiological criteria which may lead to serious outbreaks.

Purpose: Identify associations between food outbreaks and lack of microbiological criteria of food matrices.

Methods: Twenty microbiological criteria were intensively investigated. Those criteria are currently applied in fifty-three countries. According to this study approximately only one-third of food worldwide is controlled by microbiological criteria. A number of foodborne outbreaks worldwide were positively or negatively linked to microbiological criteria.

Results: Out of the microbiological criteria tested in this study, fifteen (75%) applied *E. coli* O157 testing to at least one food matrix related to meat products but not the rest. All the evaluated microbiological criteria (100%) accepted prevalence of *E. coli* in two out of five replicates of tested food samples with maximum of $5x10^2$ colony forming unit (CFU) (with no further serotype identification). For example, Brazil and India's microbiological criteria do not require testing *E. coli* O157 in food matrices. Brazil and India are two of the biggest meat importers to the Saudi Arabian market. According to Saudi Food and Drug Authority (SFDA), at least 6% of tested meat imported from Brazil and India are infected with *E. coli* O157. This is probably due to the lack of Brazil and India's microbiological criteria in testing *E. coli* O157 in food matrices before export.

Significance: There is a possible link between the absence of testing *E. coli* O157 in microbiological criteria and existence of the same pathogen in food matrices that are publicly available for consumption.

P1-38 Health Canada Risk Analysis of Shiga Toxin-Producing *Escherichia coli* (STEC) in Canada: STEC and Food Commodities of Concern

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) are a significant cause of foodborne illness in Canada. Historically, the public health measures, surveillance and risk management, have targeted STEC-O157:H7/NM due to their role in foodborne outbreaks. Illnesses associated with non-O157 STEC have increased over time in Canada.
Purpose: Health Canada's STEC risk analysis defines STEC that pose a risk in Canada and provides information on food categories to guide changes to Canadian standards.
Methods: A review of clinical and surveillance data from Canadian regulatory agencies, including Public Health Agency of Canada, was conducted to identify the specific STEC that represent hazards. Also, food commodities associated with clinical illness were identified, classified using a five-level hierarchy scheme, and ranked using a criterion that considered the frequency of the outbreaks and the number of cases.
Results: Canadian studies on the presence of virulence factors in clinical isolates show great variability in the *stx* type/subtypes. The presence of *eae* was reported in most isolates. Similar to what is observed internationally, clinical cases in Canada are associated with *stx* and *eae* regardless of their serotype. Data from Canadian sentinel sites showed that 70% of STEC cases visited the ER and most reported bloody diarrhea, indicating a high probability of severe symptoms. Our analysis of Canadian outbreaks revealed that raw ground beef (40 outbreaks, 678 illnesses) and fresh leafy greens (15 outbreaks, 285 illnesses) were most likely to be involved in STEC foodborne infections, followed by cheese made from unpasteurized milk (4 outbreaks, 64 illnesses), intact raw pork (3 outbreaks, 89 illnesses), flour (2 outbreaks, 36 illnesses) and fresh bulbs (1 outbreak, 224 illnesses).
Significance: The outcomes from our analysis defined STEC of interest in Canada and identified the food commodities of concern. This will help us update Canadian standards for STEC and improve public health outcomes.

P1-39 The Inactivation of *Salmonella* in All-Purpose and Almond Flour Brownies Cooked via Air Fryer Technology

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Introduction: In the past several years, *Salmonella* has been implicated in foodborne illness outbreaks related to contaminated flour and batters.
Purpose: This study was conducted to simulate the cooking process of brownies formulated with all-purpose and almond flour via application of air fryer technology to control *Salmonella* survival in contaminated flour.
Methods: A 3-serotype (enterica, montevideo, typhimurium) cocktail (~6 log CFU/g) was inoculated into both all-purpose and almond flour brownie batters and was cooked at 176.7 °C (350 °F) for 20 min in a pre-heated air fryer. Thermocouples were placed in various locations in the brownie pans to determine the temperature at specific sampling times. After removal from the air fryer, formulations were cooled for 15-min at ambient temperatures. Microbial, a_w, pH, and moisture analyses were conducted at 0, 5, 10, and 20 min. Samples were serially diluted and plated on XLD and enriched in Rappaport Vassiliadis broth when needed.
Results: The mean internal temperature after 20 min for the brownies formulated with all-purpose flour and almond flour was 102°C and 107°C, respectively, and was not significantly different ($p > 0.05$). *Salmonella* populations decreased by >5 log CFU/g and ~6 log CFU/g for both the all-purpose and almond flour formulations, respectively, when cooked for 20 min at 176.7 °C. The a_w and pH of both treatments decreased significantly ($p \leq 0.05$) over time. There was a significant difference ($p \leq 0.05$) in the approximate moisture and fat percentage across both formulations.
Significance: This study showed that air fryer technology was effective at controlling *Salmonella* in both the all-purpose flour and almond flour brownie formulations when cooked for 20 min at 176.7 °C and that air frying can be a safe and reliable alternative cooking method for at-home consumers when compared to conventional oven cooking.

P1-40 Effects of Temperature Distribution and Heat Penetration during the Steam-Air Retorting Process on the Quality Properties of Canned Whelk (Buccinum striatissimum)

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Introduction: Retorting is a typical heat treatment process at high temperatures and high pressures to extend the shelf life of food. In the industrial scale, precise temperature control is required since canned foods with different quality can be produced even within the same batch if a non-uniform temperature distribution is formed in the retort.
Purpose: To confirm the heat penetration performance of newly developed industrial scale steam-air retort equipment and its impact on process optimization and physicochemical properties.
Methods: Temperature distribution and heat penetration studies were conducted for two retort devices, Retort A (cylindrical steam-air retort) and Retort B (rectangular steam-air retort). A total of 400 g of the product, consisting of 195 g of whelk (*Buccinum striatissimum*) and 205 g of liquid juice, was filled into a can. Thermal process was performed at 108℃ for 10 min, and 122℃ for 30 min, consecutively, and then cooled to below 40℃. Temperature history on the different positions in the retort was analyzed using various thermal properties (i.e. F_0-values, f_h, f_c, j_h, j_c). Quality properties including color, texture, pH, and sensory attributes of the products were also analyzed.
Results: Retort A and B produced 2,640 and 2,688 canned products, respectively. During the holding phase, the temperature deviation of Retort A had about 3-4℃ compared to the set temperature (122℃), and the F_0-value ranged from 40.4 to 50.9 min. In contrast, Retort B maintained within 1℃ compared to the set temperature and the F_0-value ranged from 39.8 to 46.9 min. The results showed that pH, a*,b*, and sensory attributes decreased, but L* increased as the F_0-values increased.
Significance: It was confirmed that more precisely controlled retort device could produce canned foods with uniform qualities and guarantee product safety, so it suggests that the device is industrially applicable.

P1-41 Inactivation of *Bacillus* and *Geobacillus* Species in Pea-Based Milk Alternatives under UHT Processing

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❖ ***Developing Scientist Entrant***

Introduction: Although the use of plant-based ingredients is not new to the food industry, the way in which they are processed and used to produce dairy alternatives should be evaluated to verify if conventional processing is adequate to ensure microbiological stability of the newly developed recipes.
Purpose: The objective of the study was to predict the inactivation of *Geobacillus stearothermophilus*, *Bacillus licheniformis, Bacillus subtilis* in a pea-based milk alternative in UHT processing conditions.
Methods: *Geobacillus stearothermophilus* ATCC 7953, *Bacillus licheniformis* CTCPA 3107001 and a *Bacillus subtilis* strain isolated from a spoiled pea-based drink were selected as model organisms, as they are frequently identified in spoilage incidents. Inactivation experiments were conducted in a pea-based

Poster

milk formulation at 115-130 °C in duplicates for *G. stearothermophilus* spores and 97-110 °C in triplicates for *B. licheniformis* and *B. subtilis* spores.

Results: Predictive models were developed and reduction times in the UHT processing range, 130-150 °C, were simulated for each microorganism. The Z_T-values ranged between 8.80-9.99 °C for the three species. The time for 4 log-cycles reduction of *G. stearothermophilus* at 135 °C was 20.14s, suggesting a potential challenge for the conventional processes. For *B. licheniformis* and *B. subtilis,* the predicted times were 0.10 and 0.24 s, respectively, indicating that the established processes can sufficiently control these species. The latter is important as these species are highly prevalent in plant-based raw materials and often associated with spoiled products.

Significance: To our knowledge, this is the first study to report inactivation of spoilage species in a plant-based matrix under UHT processing. These results can be combined with prevalence data in raw ingredients and in-factory practices to assess spoilage risk and support the design of thermal processes.

P1-42 Mathematical Modeling of *Salmonella* Inactivation in Humidity-Controlled Apple Drying Process

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Introduction: Traditional food drying techniques introduce uncertainty in microbial elimination. Research is needed to develop reliable means for thermal inactivation in drying operations.

Purpose: Examine the impact of high humidity treatment on thermal inactivation of *Salmonella* during apple drying and develop a mathematical model based on the monitored process parameters to predict *Salmonella* reduction.

Methods: Fresh-cut apple cubes (6 mm) were inoculated with a cocktail of three *Salmonella enterica* strains (Enteritidis PT30, Montevideo 488275, and Agona 447967). Subsequently, the samples were placed in a preheated sample treatment box inside a convection oven under three different arrangements to simulate different pre-drying conditions: 1) box was left open, the oven was set at 90°C (Open-90); 2) box: closed, oven: 90°C (Close-90); 3) box: closed, oven: 70°C (Close-70). When closed, heat conducts into the box through the walls; internal air convection causes sample heating and moisture evaporation. When opened, hot air from the oven entered the box, and a portion vented naturally through an exhaust hole. Air temperature, relative humidity (RH), and sample temperatures were monitored, with survival populations of *Salmonella* assessed at five time-points. Experiments were triplicated.

Results: **T**ime to achieve a 5-log reduction of *Salmonella* occurred much faster in closed-box conditions (Close-90: 9 min, Close-70: 15 min), compared to Open-90 (26 min). The closed-box heating elevated the maximum air RH from 22% (Open-90) to 60% (Close-90) and 71% (Close-70). Maximum sample temperature during the constant drying rate period (dewpoint temperature) increased from 63°C (Open-90) to 72°C (Close-90). A universal model was developed that accurately predicted *Salmonella* inactivation in all conditions (RMSE=1.2 logCFU/g), with temperature and RH at the sample surface identified as key parameters.

Significance: This study underscores the pivotal role of air humidity in bacterial thermal death during drying, suggesting controllable measures for microbial reduction in food products. It also demonstrates the ability of mathematical models to predict real-time microbial inactivation in food drying processes.

P1-43 Impact of Supercooling Preservation on Salmon Quality Using Combined Electric and Magnetic Fields

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❖ ***Developing Scientist Entrant***

Introduction: Fish, being highly perishable, are prone to spoilage and oxidation. As a food preservation technology, supercooling has the potential to maintain the quality attributes of foods since the damage caused by ice formation can be completely avoided.

Purpose: This study aims to investigate the impact of supercooling preservation with electric field (EF) and magnetic field (MF) on salmon quality during a 10-day storage period.

Methods: Farm-raised salmon filets (~200 g) were in a supercooling system, and the EF and MF were applied during the entire experiment. The temperature of supercooled samples was maintained at -3 °C. The EF and MF-treated salmon samples were preserved for 10 days, and quality changes such as total aerobic plate counts (APC), thiobarbituric acid-reactive substances (TBARS), drip loss, color, and texture were measured and compared to samples subject to refrigeration and freezing conditions.

Results: The TBARS value of refrigerated salmon was significantly higher than frozen and supercooled samples after 10 days of storage ($P < 0.05$). Specifically, the TBARS value of samples after 10 days of storage showed 2.13, 0.96, and 1.06 mg MDA/ kg refrigerated, frozen and supercooled, respectively. Supercooling demonstrated the most effective control over bacterial growth in salmon over 10 days, maintaining a lower amount of APC (5.30 log CFU/ml) compared to refrigerated (7.09 log CFU/ml) and frozen (5.96 log CFU/ml) samples, as indicated by significantly lower values throughout the storage. The supercooled samples showed significantly lower values in drip loss (0.8%) compared to the refrigerated (2.5%) and frozen samples (4.1%). In addition, the color changes and texture showed that the supercooled state reduced quality degradation.

Significances: Supercooling preservation using the EF and MF combination technology allowed the salmon fillets to have a prolonged shelf-life and minimize quality degradation as other traditional storage counterparts.

P1-44 Chemical Inactivation of Spore-forming Bacteria: Simulating Acid and Alkaline Treatments from Gelatin Processing

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❖ ***Developing Scientist Entrant***

Introduction: Gelatin, derived from collagen in animal connective tissues, is vital across various industries, including food, pharmaceuticals, medical, cosmetics, and photography. Despite rigorous manufacturing conditions involving pH extremes, gelatin often retains considerable microbial counts, posing challenges to its safety and quality.

Purpose: This study investigates the effectiveness of chemical treatments, specifically acid and alkaline processes, as they occur in gelatin processing for spore inactivation.

Methods: Spore inactivation was investigated in strains resistant to gelatin processing: 3 *Bacillus cereus*, 1 *Bacillus sonorensis*, 4 *Bacillus licheniformis*, 1 *Geobacillus stearothermophilus*, 1 *Bacillus subtilis*, and 2 *Clostridium sporogenes*. Samples of porcine skin or bovine tissue were inoculated with a 6 log CFU/g spore suspension. Two pre-washing steps were conducted: washing with water and 0.1% peroxide solution for 30 minutes each at room temperature. Samples underwent acid treatment with 4% sulfuric acid for 24 or 48 hours or alkaline treatment with 1% sodium hydroxide for 7 or 10 days at 20°C. The count of survivors was performed after neutralization and heat shock. An analysis of variance followed by Tukey's test (95% confidence) evaluated the significance of observed differences.

Results: Spore reductions varied significantly depending on the strain and treatment duration. Acid treatment for 24 hours resulted in reductions from 0.57 to 2.01 log CFU/g, and 48 hours yielded reductions between 0.95 and 3.79 log CFU/g. The alkaline treatment showed reductions from 0.85 to 3.09 log CFU/g after 7 days and 1.60 to 3.68 log CFU/g after 10 days.

Significance: The study demonstrates that acid and alkaline treatments can reduce spore-forming bacteria during gelatin processing, though efficacy varies by strain and treatment duration. These findings could improve processing protocols to enhance the microbiological safety and quality of gelatin. By ensuring lower bacterial counts, the industry can better protect consumer health and strengthen the global competitiveness of gelatin products across diverse applications.

P1-45 Phage Biocontrol of Shiga-Toxigenic *Escherichia coli* on Leafy Greens

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Introduction: Mitigation of STECs on leafy greens poses a significant challenge as a limited repertoire of microbial interventions can be safely used without affecting the physicochemical and organoleptic properties of the leafy greens. Lytic bacteriophages have emerged as a natural and effective modality to reduce foodborne pathogens on foods without impacting the physicochemical and organoleptic properties.
Purpose: We tested the efficacy of a cocktail of five naturally occurring lytic bacteriophages, chosen for their efficacy against STEC serotypes, against STEC contamination of lettuce.
Methods: STEC challenge hosts (serotypes O157, O26, O45, O103, O111, O121, O145) were individually spot inoculated onto 3x3cm romaine lettuce portions at 4 log CFU/leaf. Cells were allowed to attach at room temperature for 1h before spraying with $1x10^8$ PFU/leaf bacteriophage cocktail. Samples (n=9/treatment) were stored at room temperature for 1h or at 4°C for up to 48h. Viable STECs were enumerated by direct plate count on MacConkey agar at 1h, 24h, and 48h post-phage application. Additionally, *E. coli* O157:H7 (4 log CFU/leaf) was inoculated onto whole romaine leaves (n=18/treatment) and sprayed with $1x10^8$ PFU/leaf bacteriophage cocktail before storage in sealed bags (3 leaves/bag) for up to 5 days to simulate field washing and packaging. Viable STEC were enumerated as above at 48h, 72h, and 120h.
Results: The bacteriophage cocktail significantly reduced *E. coli* populations on romaine lettuce. Phage application resulted in up to 2.1, 2.7, 2.4, 3.4, 3.3, 3.6, and 2.5 log CFU/leaf reductions in O157:H7, O26, O45, O103, O111, O121, and O145 populations, respectively. The reductions were highly significant (p<0.01). *E. coli* O157:H7 was also significantly reduced on bagged romaine by up to 2.5 log CFU/leaf (p<0.01) over 5 days.
Significance: These data suggest that bacteriophages are a highly effective mitigation strategy for STEC contamination of leafy green products when applied as a produce wash.

P1-46 The Effect of Fat Level on the Inactivation and Recovery of *Listeria* spp. in Ready-to-Eat (RTE) Foods After High Pressure Processing (HPP): A Review

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a foodborne pathogen that causes a severe infection known as listeriosis that affects approximately 1,600 individuals and causes around 260 deaths annually in the USA. Ready-to-eat (RTE) foods are particularly vulnerable to *L. monocytogenes* contamination from the environment during processing steps, such as slicing and packaging. High pressure processing (HPP) is a nonthermal pasteurization technology that has been used as a post-lethality treatment to control *L. monocytogenes*.
Purpose: Determine the effect of fat level on the efficacy of inactivating and mitigating the recovery of *Listeria* spp. in RTE foods by HPP.
Methods: A scoping review of the literature was conducted following the PRISMA-ScR reporting guidelines and the Arksey and O'Malley framework. A total of 938 journal articles involving HPP treatment of RTE meat and poultry, seafood and dairy products to control *L. monocytogenes* and *L. innocua* were screened. Microbial inactivation, recovery, and corresponding HPP and storage conditions were extracted and analyzed from 17 papers that reported fat levels.
Results: For RTE meat and poultry products (fat=1.7 to 28.8%), trends showed microbial inactivation levels increased as pressure increased, and fat levels decreased. *L. monocytogenes* recovery post-HPP treatment, however, decreased as pressure increased and as fat levels increased. Not enough data were available for (a) *L. innocua* in RTE meat and poultry products and (b) *Listeria* spp. in RTE seafood and dairy products to infer any trends.
Significance: This review shows the importance of reporting product composition in microbial challenge studies involving HPP, as fat can greatly affect the level of inactivation and recovery of *L. monocytogenes* in RTE meat products. The review also highlights the need for process validation studies involving HPP as a post-lethality treatment to include *L. monocytogenes* recovery data over the intended product shelf-life.

P1-47 Survival of *B. coagulans* Spores in Acidified Tryptic Soy Broth after High Pressure Processing

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Introduction: High-Pressure Processing (HPP) is one the most recent technologies used to process foods. It inactivates pathogenic microorganism but is not effective against microbial spores. Given this hurdle, the pH of food products must be below 4.6 for safety validation to prevent *Clostridium botulinum* from growing.
Purpose: The aim is to investigate the survival six *Bacillus coagulans* strains after heat-induced sporulation and then HPP and to examine their inhibitory effects against pathogens using deferred inhibition zones.
Methods: Six *B. coagulans strains* were selected, four isolated from commercial kombucha and two were from USDA ARS Culture Collection. They were grown in acidified Tryptic Soy Broth (4.6<pH<7.2), sporulated, processed at 600 MPa for 3 minutes. were tested for heat-induced sporulation and HPP survival. *B. coagulans* antimicrobial properties were tested against the following organisms *C. sporogenes, C. perfringens*.
Results: Results showed that *B. coagulans* spores isolated from kombuchas survived HPP (less 0.5 log reduction) when the pH is above 4.9. *B coagulans* NRS-609 (USDA strains) was below the detection limit after HPP treatment with all the pH tested. Overall, *B. coagulans* strains displayed inhibition zones against the tested strains indicating their potential as protective culture.
Significance: These results highlight the survival of *B. coagulans* spores in low acid media after HPP. Additionally, the strains ability to inhibit *Clostridium sporogenes* and *Clostridium perfringens* suggests their potential as protective cultures in low acid beverages.

P1-48 Effects of High Hydrostatic Pressure Treatment on Aromatic Amino Acids, Biogenic Amines, and Bacterial Diversity of Stinky Tofu

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Introduction: The stinky tofu is made by a spontaneous fermentation of the brine containing a mixture of vegetables, meats or herbs to obtain a unique and strong stinky odor. However, it's easily contaminated by environmental pathogenic bacteria during the fermentation process, and the quality is difficult to control.
Purpose: The goals of this study were to determine the effect of high hydrostatic pressure (HHP) treatment on the aromatic amino acids, biogenic amines content and bacterial diversity of stinky tofu.
Methods: The stinky tofu treated at 0.1(control group), 300 or 600 MPa for 5 minutes were refrigerated at 4°C for 60 days. Samples were taken on days

0, 7, 15, 30, 45 and 60 to determine aromatic amino acid and biogenic amine contents, physiochemical properties, and bacterial diversity.

Results: The dominant bacterial genera in stinky tofu are *Leuconostoc, Lactobacillus, Kurthia, Lactococcus,* and *Pediococcus.* The bacterial diversity of stinky tofu is significantly reduced after HHP treatment, which also inhibits the growth of pathogens *(Pseudomonas* and *Staphylococcus)* and increases the proportion of pressure-resistant bacteria *(Bacillus).* The total bacterial count and fungal count was also significantly reduced by HHP, which contributes to the microbial safety of stinky tofu in cold chain circulation. On the 60th day, the contents of biogenic amines (spermidine, cadaverine, and putrescine) in stinky tofu treated with 600 MPa were 30-47% lower than those in the control group. In the aroma analysis, the phenol, p-cresol, and indole contents of the control group samples were 42.5 μg/mL, 114.6 μg/mL, and 158.2 μg/mL respectively, which were no different from those of the HHP treatment group.

Significance: HHP treatment inhibits the growth of pathogenies in stinky tofu and reduces the accumulation of biogenic amines but does not affect its original fermented flavor. This technology can reduce the microbial risk of stinky tofu and extend the shelf life.

P1-49 Effects of High-Pressure Assisted Enzyme Penetration Treatment on Acrylamide Mitigation in Sweet Potato Fries

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Introduction: Acrylamide is present in foods subjected to processes at temperatures above 120°C, and it is formed through the reaction between asparagine and reducing sugars in the Maillard reaction.

Purpose: The effects of high-pressure processing (HPP) assisted asparaginase penetration treatment for the reduction of acrylamide content in sweet potato fries were studied.

Methods: Raw sweet potatoes were immersed in asparaginase (10,000 ASNU/L) solution and immediately treated at 200, 400, and 600 MPa for 5 min. The total reaction time of asparaginase was 10, 15, and 20 minutes. Samples were then evaluated for changes in the weight, moisture, water activity, reducing sugars and asparagine content of raw sweet potato in different HPP treatments. Sweet potato fry samples were analyzed for hardness, browning degree and acrylamide production after frying at 190°C.

Results: The results found that HPP pretreatment can reduce the asparagine content by 12-39% in raw sweet potato. Compared with untreated samples, raw sweet potatoes treated at 600 MPa with the immersed of asparaginase reaction for 15 minutes can achieve a 45% reduction in acrylamide content of sweet potato fries, while HPP treatment or asparaginase reaction alone cannot significantly reduce acrylamide content. In addition, it was also found that during the asparaginase soaking process of raw sweet potatoes, reducing sugars (fructose and glucose) will be dissolved into the soaking liquid, which may decrease browning index and color change (ΔE) of sweet potatoes after frying. HPP treatment also slightly reduced the moisture content and water activity of raw sweet potato, and the hardness and crispness of sweet potato fries were significantly improved, but the degree of weight loss was significantly higher than that of the control group.

Significance: HPP-assisted asparaginase penetration treatment can effectively reduce the formation of acrylamide in sweet potato fries, allowing consumers to consume healthier fried foods without changing their eating habits.

P1-50 Emerging Topics of High-Pressure Processing: Improving Efficacy of DNA and Mycotoxins Extraction and Biofilm Formation of Pressure-Stressed Bacteria

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Introduction: Adaption of high-pressure processing technologies in various sectors of food and pharmaceutical industries are continuing to gain importance and momentum as an efficacious non-thermal processing with high consumers' acceptability.

Purpose: This oral presentation discusses recently completed studies in the Public Health Microbiology program in Nashville for improving the sensitivity of culture-independent detection of bacterial pathogens and mycotoxins. The presentation will additionally discuss the importance of pressure-stressed bacterial pathogens and their biofilm formation capability.

Methods: Various studies conducted using Hub440 and Hub880 Barocycler Units (110 to 650 MPa, 4.4 to 60.0 °C), a lateral flow immunochromatographic platform for quantification of aflatoxin and ochratoxin, and standard and real-time PCR assays using BAX system Q7 were statistically analyzed using Tukey-adjusted ANOVA with type I error level of 5%. Studies involving biofilm formation of pressure-stressed bacterial pathogens and surrogate microorganisms on abiotic surfaces (type 304, #2b finish stainless steel coupons) will additionally be discussed.

Results: Results of recently completed challenge studies illustrate that repeated cycles of high-pressure processing (30 cycles of 240 MPa for 20 seconds/cycle) could increase ($p<0.05$) sensitivity of standard and real-time PCR assays and could be considered as an alternative for selective enrichment of samples with low presence of bacterial pathogens. Additionally, same treatment cycles could augment the extraction efficacy of mycotoxins of public health concern. Our results additionally illustrate the importance of pressure-stressed bacterial pathogens with sublethal pressure injuries and their capability to proliferate and form sessile communities, comparable to wild-type cells.

Significance: Results of recent studies illustrate that elevated hydrostatic pressure could be of great assistance in detection of bacterial genes in culture-independent analyses, with particularly important implications for real-time PCR quantification of microorganisms. Additionally, the application of pressure processing could be of great importance for improving the extraction of mycotoxins of public health concern from various commodities.

P1-51 Impact of Elevated Hydrostatic Pressure for Improving Extraction and Inactivation of Fungal Secondary Metabolites of Public Health Concern

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✦ ***Undergraduate Student Award Entrant***

Introduction: Mycotoxins, including aflatoxin and ochratoxin, are secondary metabolites of fungal origin and their ingestion is linked to deleterious health consequences for humans and animals.

Purpose: The current study (a) investigated the prevalence of these two secondary metabolites in dried commodities, (b) investigated impact of high-pressure processing for improving extraction/detection of the compounds, and (c) investigated impact of elevated hydrostatic pressure for inactivation of aflatoxin B1 and ochratoxin.

Methods: Using a lateral flow immunochromatographic platform, presence of aflatoxin and ochratoxin in dried commodities were quantified. Naturally contaminated and artificially inoculated powdered milk and brewed coffee were then exposed to elevated hydrostatic pressure of 650 MPa at 4 and 60 °C for 3 to 30 minutes to study the impact of treatments on improving detection/extraction of the metabolites and their inactivation. The replicated trials were analyzed by analysis of variance (type-I error level of 5%).

Results: Among tested products, 86.7% and 13.3 % were positive for aflatoxin and ochratoxin, respectively with the highest concentration associated with a ground spice (42.5 ± 4.5 ppb of aflatoxin). Cycles of elevated hydrostatic pressure (30 cycles of 20-second pressure treatment at 240 MPa), were effective ($P < 0.05$) to increase the recovery of both natural and artificially added mycotoxins. Up to 4.47 and 31.40 ppb of additional aflatoxin in naturally and artificially contaminated dried milk were detected, respectively as results of incorporation of pressure treatments during the extraction procedure. Treatments of 650 MPa at 60 °C for up to 30 minutes did not result ($P \geq 0.05$) in decomposition of the mycotoxins.

Significance: High pressure processing exhibited promising results for improving extraction and detection, for more accurate quantification of these fungal compounds of public health concern. Additionally, our results show the importance of preventive measures against formation of mycotoxins in food commodities due to their resistance to non-thermal treatments.

P1-52 Combining Lactic Acid Dipping and Mild High-Pressure Processing for the Inactivation of Non-Pathogenic *Enterococcus faecium* as a *Salmonella* Surrogate in Pork

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❖ ***Developing Scientist Entrant***

Introduction: Hurdle technology is a common decontamination process in the meat industry. High-pressure processing (HPP), as cold pasteurization, could be used in combination with current organic acid dipping to synergistically reduce *Salmonella* contamination in pork products while maintaining the sensory quality.

Purpose: Explore the synergistic impact of mild HPP (pressure and time) and lactic acid (concentration) on inactivating non-pathogenic *Enterococcus faecium* NRRL B-2354 (surrogate for *Salmonella*) in pork.

Methods: Central Composite Rotatable Design with three center points and α of 1.68 was utilized to systematically evaluate the effects of three parameters: pressure (100-300 MPa), time (1.5-5 minutes), and acid concentration (1.5-4% w/w)], across 17 treatment combinations. Two levels of fat content were evaluated - lean pork loin (95/5) and belly (60/40). For each fat content, one group of samples was inoculated to a concentration of 7 log CFU/g and subjected to a lactic acid dip before HPP treatment. A second group, comprising uninoculated samples, was similarly processed for the color analysis. Inoculated samples were enumerated for microbial load 24 hours post-treatment. Polynomial regression models were built to evaluate the impact of HPP and acid treatment conditions on microbial log reduction.

Results: The log reduction across all treatment combinations ranged from 0.9 to 2.6 log CFU/g. All factors showed significant linear effects ($p<0.05$) on the reduction of *E. faecium* in pork meat, with pressure additionally demonstrating a quadratic effect. The influence of pressure was the most pronounced, followed by time and acid concentration. The R^2 for models was 0.65 for low fat and 0.74 for high fat samples with a non-significant ($p>0.05$) lack of fit, indicating the models were well-fitted.

Significance: Results of this study could potentially be used as guidelines for HPP application as an additional hurdle to the current acid dipping process for pathogen intervention in raw pork meat.

P1-53 Combined Effect of High Hydrostatic Pressure (HHP) and Nisin against Five Strains of *Listeria monocytogenes* in Aces Buffer

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❖ ***Developing Scientist Entrant***

Introduction: Over the past few decades, High Hydrostatic Pressure (HHP) has emerged as an innovative non-thermal sustainable technology, able to inactivate spoilage and pathogenic microorganisms ensuring food safety and extending shelf life of food products. Even though HHP is used in the food industry, its cost is relatively high. Combination of HHP with nisin increase efficacy and reduce costs by reducing pressure intensity.

Purpose: The primary objective is to investigate the potential synergistic or additive effect of HHP and nisin combination against piezotolerant and piezosensitive *L. monocytogenes* strains optimizing the HHP technology.

Methods: This work presents the combined effect of different pressures (200–350 MPa) with different concentrations of nisin (100, 250 & 500 IU/mL) on the inactivation of five *L. monocytogenes* strains (2 piezotolerant, 2 intermediate & 1 piezosensitive). Cultures were grown in nutrient rich medium to stationary phase. Centrifugation and resuspension in ACES buffer were carried out before the nisin application and the HHP treatment.

Results: *L. monocytogenes* cells were reduced by 1.7 - 4.0 log CFU/mL (depending on the strain) combining HHP (300 MPa) and 500 IU/mL of nisin. No significant strain variability was observed in the cell reduction due to nisin application for 1h. The highest synergistic effect was observed on FBR13. More than 5 log reduction was achieved against the most piezotolerant strain (L6) combining nisin and HHP (350 MPa).

Significance: HHP and nisin combination was very effective against *L. monocytogenes* in ACES buffer regardless the strain. Subsequently, nisin could be considered as a potential natural antimicrobial which combined with HHP can achieve safe food products, optimizing the HHP technology. The results of this research can also be used for validation studies in HHP with nisin treated food products.

P1-54 Unraveling the Synergistic Lethal Effect of Manothermosonication under Dynamic Conditions on the Microbial Resistance of *Salmonella* Enteritidis in Liquid Whole Egg

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Introduction: Manothermosonication (MTS) offers a promising alternative to traditional thermal pasteurization of liquid whole egg (LWE) aiming to reduce thermal impact and minimize effects on quality.

Purpose: This work investigated the efficacy of MTS in inactivating *Salmonella* Enteritidis DSM 17420 in LWE by modeling the inactivation kinetics under dynamic thermal conditions and evaluated its synergistic lethal effect by comparing it with the isolated effects of ultrasound and dynamic heat.

Methods: A DIL-designed MTS prototype was employed using a central composite face-centered design with wave amplitude (66-132 µm) and pressure (100-300 kPa) as processing parameters to assess the inactivation kinetics. Kinetic parameters (Dref-, zT-, and zUI-values) were estimated by modeling the microbial inactivation under dynamic conditions at reference conditions (57°C, 166.91 W/cm^2) and using a log-linear and an extended Bigelow models accounting for the microbial resistance to the ultrasound intensity. Additionally, inactivation kinetics for temperature-controlled ultrasound and dynamic thermal treatments were modeled, providing respective kinetic parameters.

Results: Results indicated that higher amplitude (132 µm) resulted in increased microbial inactivation, while elevated pressure (300 kPa) did not consistently maximize inactivation rates. However, higher ultrasound intensity (related to amplitude and pressure) led to a fast temperature increase, impacting microbial sensitization, and resulting in higher inactivation in shorter times. The estimated Dref-, zT-, and zUI-values were 1.12 ± 0.01 min, 22.93 ± 0.02°C, and 692.52 ± 0.02 W/cm^2, respectively. MTS exhibited synergistic inactivation, with Dref-values of 13.03 ± 0.39 and 5.56 ± 0.23 min for ultrasound and dynamic thermal treatments, respectively.

Significance: MTS emerges as an alternative to traditional LWE pasteurization, requiring the same processing time (3.5 min) under dynamic conditions (final temperature of 57°C) to achieve the same *Salmonella* spp. inactivation safety level (5.0 log10 CFU/mL reductions) as isothermal treatments at 60°C. Consequently, MTS demands lower thermal exposure, resulting in a milder LWE treatment.

P1-55 Microbial Strain Heterogeneity to Pulsed Electric Fields (PEF) Treatments

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❖ ***Developing Scientist Entrant***

Introduction: Pulsed Electric Fields (PEF) is an emerging alternative to thermal processes technology for food preservation, which stands out for its minimal impact on nutritional and sensory characteristics. The application of PEF for microbial inactivation is based on high electric field pulses of a short duration (from micro - to milli-seconds) with an electric field strength of 15 - 40 kV/cm.

Purpose: In this study, we investigated the impact of PEF on microbial inactivation across 40 strains of four model microorganisms: *Escherichia coli, Listeria monocytogenes, Lactiplantibacillus plantarum, and Saccharomyces cerevisiae*.

Methods: Employing an electric field strength of 20 kV/cm and varying total specific energies (88, 136, and 184 kJ/kg) on pH 7.0 microbial samples, strain-specific resistances and PEF treatment correlations were investigated.

Results: The findings revealed diverse strain-specific responses among the microorganisms relative to the total specific energy used. *Escherichia coli* strains exhibited notable $\log_{10}$ inactivation disparities under 88 and 136 kJ/kg treatments, contrasting *L. monocytogenes* strains that displayed significant differences ($p<0.05$) solely under the 184 kJ/kg treatment. Particularly, *L. monocytogenes* L6 strain emerged as the most robust-PEF resistant, demonstrating a 1.23 $\log_{10}$ cycles reduction under the highest PEF treatment. On the opposite, under the highest PEF treatment condition, 184 kJ/kg, *E. coli* strains showed no statistical differences ($p<0.05$) and a log reduction ≥ 4.04.

Significance: Identifying resilient strains is pivotal for tailoring PEF treatment parameters or combining methods to ensure food safety, showcasing the intricate variability among species and strains under specific PEF conditions. These insights underscore the necessity of technology-specific assessments to gauge resistance levels in microorganisms crucial for public health, vital for designing precise and effective PEF treatments and enhancing food safety standards.

P1-56 Cold Plasma Treatment Distance Influences Reduction of *Salmonella enterica* on the Surface of Pecan Halves

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Introduction: Tree nuts are often eaten raw, presenting a potential risk for foodborne pathogen outbreaks. Cold plasma has been proposed as a non-thermal alternative to other sanitation methods for some tree nuts but has not been thoroughly investigated for use with pecans.

Purpose: The objective of this study was to determine the efficacy of cold plasma at various distances to reduce *Salmonella* at varying microbial loads on pecans halves at different orientations.

Methods: Individual pecan halves were individually spot inoculated on the interior or exterior surface of the kernel with 40 ul of a high (≈ 9 log CFU/ml) or low (≈ 7 log CFU/ml) 5-strain rifampicin resistant *Salmonella enterica* cocktail. Samples were treated in triplicate with cold plasma at a frequency of 500 kHz at distances of 4cm or 8cm from the emitter for 10 seconds. Samples were transferred to stomacher bags, diluted with 0.1% peptone, and homogenized. Samples were plated on to tryptic soy agar supplemented with rifampicin and enumerated. Data was analyzed by ANOVA and Tukey's HSD ($p\leq0.05$).

Results: Microbial reduction (log CFU/g) was significantly different ($p<0.05$) between pecans treated at 4 cm compared to 8 cm. Exterior-inoculated pecans treated at 4 cm had significantly higher reductions for both inoculation levels (1.18±0.39 and 1.14±0.39 log CFU/g high and low inoculum respectively) compared to pecans treated at 8 cm (0.78±0.31 and 0.51±0.23 log CFU/g high and low respectively). Likewise, interior-inoculated pecans treated at 4 cm had significantly higher reductions (1.18±0.30 and 0.94±0.40 log CFU/g high and low inoculum respectively) compared to pecans treated at 8 cm (0.65±0.40 and 0.48±0.24 log CFU/g high and low respectively). Pecan orientation (exterior vs interior) and inoculation level (high and low) did not significantly influence *Salmonella* reduction.

Significance: Cold plasma efficacy is not inhibited by structural differences of pecan halves, nullifying concerns regarding orientation during treatment, with distance being a significant factor influencing *Salmonella* reduction.

P1-57 Effect of Cold Atmospheric Plasma on the Nutritional Properties, Texture and Color of Ready-to-Eat Ham, Salmon, and Cheese

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Introduction: The implementation of emerging non-thermal technologies by food industry during last decade has been studied as new alternative for processing and product shelf-life extension. In this case, cold atmospheric plasma (CAP) is a technology composed by positive and negative ions, free radicals, UV emission, reactive oxygen and nitrogen species, among others, that can prevent food deterioration during storage.

Purpose: The objective of this study was to determine the effects of CAP on the nutritional composition (total fat, protein, moisture, ashes), texture and color attributes of ready-to-eat foods (Gouda cheese, pork ham and cold smoked salmon) during storage time.

Method: CAP treatments were applied directly and indirectly, for 15, 30, 60, 90 and 120 s using a gliding arc plasma jet device After CAP application to the samples, shelf-life studies were carried out (days 1, 4, 7 and 10) for nutritional composition. Color was evaluated in L* a* b* coordinates and ΔE, and texture through instrumental compression profiling.

Results: The study showed that there were no significant differences in moisture and ashes parameters ($p>0.05$), while fat and protein contents showed some changes ($p<0.05$) in the foods studied. The CAP treatments of 90 and 120 s showed significant reduction on protein in ham and salmon, while fat content significantly increased and decreased in pork ham and smoked salmon respectively. Firmness properties in CAP treated Gouda cheese showed higher values at the end of its shelf life. Color was not affected in CAP treated samples.

Significance: CAP presented minimal effects on nutritional properties, texture, and color. Therefore, CAP is a novel non-thermal technology that can effectively maintain food quality and extend shelf life without significantly affecting nutritional properties.

P1-58 Enhancing Microbial Safety of Almond Milk Using UV-C Irradiation

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Introduction: The beverage industry faces significant challenges in preventing food contamination by pathogenic microorganisms. Thermal pasteurization effectively reduces the population of pathogenic and spoilage microorganisms but causes biochemical changes affecting product quality and hence there is a need for non-thermal pasteurization technologies.

Purpose: The present study evaluated the effects of UV-C irradiation on microbial reduction and shelf life of almond milk (AM) at refrigeration and abusive temperatures.

Methods: We designed a continuous Dean flow UV-C system using fluorinated ethylene propylene tubing (UV transmission >60%), arranged in a

serpentine pattern to facilitate axial mixing (Dean number>140). The system's efficacy in inactivating *Salmonella* Typhimurium ATCC 13311, *Saccharomyces cerevisiae* ATCC MYA-4941, and T1UV was tested first. These microorganisms were inoculated in AM (10^7 CFU/ml) and exposed to varied UV-C fluence levels (0-35 mJ/cm^2) at a constant flow rate of 515 ml/min, in three biological replicates. Additionally, a shelf-life study was conducted on AM inoculated with 10^5 CFU/ml of *Salmonella* Enteritidis ATCC 4931, treated under similar experimental conditions, and stored at 4±1°C and 10±1°C for 28 days. Growth rates were obtained and modeled using DMFit program.

Results: *S.* Typhimurium ATCC 13311, *S. cerevisiae* ATCC MYA-4941, and T1UV were reduced by >4 log/ml in AM. Computational fluid dynamics (CFD) used to analyze flow velocity and irradiation distribution, revealed that higher average velocities (3.07 m/s) were observed at bends indicating high axial mixing. Shelf-life studies revealed that UV-C irradiation was effective in extending the shelf life of AM to 28 days at 4±1°C and 10±1°C, suppressing *Salmonella* Enteritidis.

Significance: The research findings indicate that UV-C treatment can achieve significant pathogen inactivation in almond milk, demonstrating its potential for extending shelf life.

P1-59 Inactivation of Cronobacter sakazakii Surrogate after Irradiation with 222-nm and 254-nm Ultraviolet-C Light

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❖ ***Developing Scientist Entrant***

Introduction: Far-Ultraviolet-C (UVC) light (222-nm) is an emerging treatment source for the irradiation of *Cronobacter sakazakii*, a bacterium of concern in low-moisture food mainly due to its desiccation survivability, without the safety concerns posed by the more extensively studied, 254-nm UVC light.

Purpose: The present study compares the efficacy of 222-nm with 254-nm far-UVC light for inactivating *C. sakazakii* surrogate, *Enterococcus faecium*.

Methods: The inactivation of *E. faecium* was evaluated using 222-nm and 254-nm UVC treatment on tryptic soy agar yeast extract plates at 2, 5, 10, and 15 minutes to determine the sensitivity to both wavelengths. Because the 10-minute 254-nm treatment reached the limit of detection, 0.56 log CFU/mL, experiments at 15 minutes were not performed. A 40-minute temperature versus time study was performed in each chamber to determine the heat generated during treatments. All experiments had a minimum of three replications. Statistical analysis was performed using a student t-test at $p<0.05$.

Results: For the 254-nm treatment, exposure times of 2, 5, and 10 minutes resulted in reductions of 7.6± 0.3, 7.8± 0.3, and 8.2± 0.04 log CFU/mL with the limit of detection reached for two samples at 5 and 10 minutes. For the 222-nm treatment, exposure times of 2, 5, 10, and 15 minutes resulted in reductions of 4.8± 0.6, 6.1± 0.2, 6.3± 0.2, and 6.6± 0.3 log CFU/mL. Each 254-nm and 222-nm treatment of comparable time significantly differed from the other. The temperature of the 254-nm and the 222-nm chambers increased from 22.87°C± 0.72 and 22.77°C± 0.31 to 39.93°C± 0.21 and 35.73°C± 0.21 after 40 minutes of treatment.

Significance: This study provides evidence for the inactivation efficacy of 222-nm far-UVC light against C. sakazakii through the measured reduction of its surrogate, Enterococcus faecium. While the inactivation was lower in the 222-nm than in the 254-nm treatment, the differences in temperature and intensity provide a possible explanation for discrepancies in log reduction.

P1-60 Efficacy of Plasma-Activated Water in Cell Membrane Damage and *Salmonella* Inactivation in Egg Washing

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Introduction: Plasma-activated water (PAW) is an emerging sanitizer for food/food contact surfaces.

Purpose: The purpose of this study was to investigate microbial inactivation mechanism by PAW and its use in egg washing.

Methods: PAW was generated by exposing deionized water (DI) to cold atmospheric pressure plasma. An avirulent *Salmonella* Typhimurium MHM112 was treated with PAW or DI for 3 min at 40 - 46 °C. After centrifugation and resuspension in 1 x sterile phosphate buffer, it was stained with propidium iodide, followed by fluorescent intensity measurement using a micro-plate reader. To test the efficacy of PAW on egg surfaces, eggs inoculated with *Salmonella* were gently massaged in 150 mL PAW or DI at 40 - 46 °C for 3 min. Eggs were then hand massaged in 20 mL buffered peptone water and diluted and plated onto tryptic soy agar (TSA). The spent solutions of PAW or DI were plated onto TSA. The spent solutions were reused to treat newly inoculated eggs and steps were repeated for egg and spent solution analysis. Both studies were performed in triplicate (student's t-test at $p<0.05$).

Results: The average fluorescent intensity (hence the cell membrane damage) for PAW was higher than DI but this difference was insignificant (p-value: 0.1039). DI and PAW caused 1.9-2.9 log reductions in egg washing as well as when spent solutions were reused for egg washing. PAW did not have any surviving *Salmonella* (<0.5 log CFU/egg) the first time but when analyzed after reusing it, PAW had surviving *Salmonella*. However, DI spent had surviving *Salmonella* after both cycles of egg washing.

Significance: DI and PAW at 40 - 46 oC can cause cell membrane damage of *Salmonella*. PAW and DI detach bacteria during egg washing but bacteria do not survive in PAW while survivability in DI can lead to cross contamination.

P1-61 Evaluation and Comparison of Plasma-Activated Water (PAW) as a Sanitizer in Cleaning-in-Place (CIP) with Other Traditional Cleaning Solutions

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❖ ***Developing Scientist Entrant***

Introduction: Water treated with air plasma (PAW) was selected as a CIP cleaning solution due to its acidic pH, and ability to inactivate bacteria and biofilms.

Purpose: This study investigated PAW as a more sustainable alternative to sanitizer in a traditional CIP.

Methods: Type 304 stainless steel coupons were inoculated with mixed strains of *E. coli* O157:H7 and *Listeria innocua* in equal proportions to grow biofilms for 24 hours at 37 °C. Whey and pea protein-based fouling deposits were formed on coupons. Inoculated fouled stainless-steel coupons were treated with different CIP chemicals along with PAW for 5 minutes at a velocity of 0.22 m/s using a continuous piping system at 45 °C. The inactivation efficacy of PAW and traditional CIP chemicals (alkaline, acid, RO water, and sanitizer) was determined in 3 independent replications and analyzed using a student t-test at $p<0.05$.

Results: The result showed that PAW alone reduced biofilm on whey and pea protein deposits by 4.2 and 3.0 log CFU/coupon, while traditional CIP sanitizer achieved 1.8 and 3.2 log CFU/coupon, respectively. Using an alkaline solution on biofilms from both protein source deposits resulted in more than 5 log CFU/coupon reductions. Moreover, traditional acid solution removed biofilms by 2-3 log CFU/coupon, whereas RO water removed biofilms by more than 2 log CFU/coupon. While most of the other spent cleaning solutions did not contain any detectable survival bacteria (<1 log CFU/mL), the RO water spent wash solution had survival bacteria (>2.5 log CFU/mL).

Significance: This study showed that PAW is an ideal solution as compared to sanitizer to remove biofilms in the presence of fouling deposits in CIP.

P1-62 Effects of Continuous UV, Pulsed Light, and LED Treatments on Inactivation of *Escherichia coli* ATCC 25922

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Introduction: Light irradiation techniques, such as continuous ultraviolet (CUV), pulsed light (PL), and light-emitting diodes (LEDs), have gained attention as non-thermal methods for surface decontamination in the food industry. More fundamental study is needed to compare these techniques.
Purpose: To understand the characteristics of CUV, PL, and LEDs and their impacts on the microbial inactivation.
Methods: Light irradiation was conducted using self-designed equipment with various types of light sources, including UV lamps, a xenon flash lamp, and LEDs. Emission spectra and fluence rates were measured by spectroradiometers. Light energy was treated onto the surface of solid agar (TSA media) or bacterial suspension (diluted in saline). During each treatment process, the log reduction of *Escherichia coli* ATCC 25922 (initial population of 10^6 CFU/mL) was monitored using the plate count method, and the obtained survival curves were fitted with microbial inactivation models.
Results: Three light irradiation techniques exhibited distinct energy properties depending on the light sources and treatment conditions. The inactivation efficiencies were significantly different with the applied wavelengths (p<0.05). Total fluences required for 5-log reductions were 4.01 mJ/cm^2, 3.60, 43.54, 56.45, 120.88, and 188.99 J/cm^2 with UV-C, UV-B, UV-A lamps, 365 nm, 385 nm, and 410 nm LEDs, respectively. Differences in inactivation kinetics were also observed. In the results of curve fitting, the modified log-linear model was determined to be the best-fitted inactivation model for the UV-C, UV-B lamp, 265 and 280 nm LED treatments (R^2>0.98), while the double Weibull model was considered suitable for the UV-A lamp, 365, 385, 410 nm LED and PL treatments (R^2>0.99).
Significance: It would be a novel approach to compare the disinfection performance between CUV, PL and LEDs. The comparative studies may provide new insights for advancing the light-based microbial control technology.

P1-63 Development of Visible Light-Induced Antimicrobial Materials for Ultrafast Inactivation of Microbes

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Introduction: Addressing the challenges of antimicrobial resistance of microbes requires the development of novel approaches to manage microbial risks in food and agriculture systems. Many alternative solutions based-on either bio or chemical approaches lack efficacy compared to conventional antimicrobials in terms of the rate and level of inactivation and thus have not been widely adopted in the industry.
Purpose: This research focused on developing novel visible-light-active solid matrices modified with bio-affinity ligands to rapidly inactivate a wide range of target microbes. This approach addresses many of the challenges of conventional photosensitizers and develops novel antimicrobial materials that can be used for diverse applications.
Method: Curcumin-modified nanofibrous membranes and zein membranes were functionalized with yeast cell wall particles as a bio-affinity ligand for capturing diverse pathogens. The increased localization of target microbes in the vicinity of the photoactive species enhanced the antimicrobial activity of generated reactive oxygen species upon light exposure. The experimental validation of affinity ligands' role in enhancing the antimicrobial performance and inactivation rate was investigated against *Escherichia coli* O157:H7, *Listeria innocua, Candida albicans* and bacteriophage T7.
Results: The bio-functionalized matrices showed a high affinity for binding the diverse microbial targets and significant enhancement in generated reactive oxygen species using visible-light. This combined effect resulted in a 15 to 30-fold increase in the inactivation rate of tested bacterial, fungal, and viral targets compared to conventional photosensitizer materials. Using visible-light-induced ROS, these novel materials achieved more than 7 log (99.99999%) inactivation of the target microbial population in a short treatment time ranging between 2-5 min. These novel materials could be reused for multiple cycles, achieving significant inactivation for at least seven consecutive treatment cycles.
Significance: This work illustrates the development of the next-generation of light-induced materials for ultrafast inactivation of diverse microbes. Applications of these materials in food and agricultural systems can enhance food safety and reduce the risk of contamination from food contact surfaces.

P1-64 Inactivation of Indigenous Microorganisms on Lettuce (*Lactuca sativa*) and Sesame Leaves (*Perilla frutescens*) Using Intense Pulsed Light (IPL) Technology

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Introduction: Intense pulsed light (IPL) is a powerful light that emits a strong light in the range of 170-1100 nm, for a very short time. This technology can extend the shelf life of food by controlling pathogenic and spoilage microorganisms, such as bacteria, yeast, and mold, present on the surface of the food.
Purpose: The microbial inactivation effect of the IPL on indigenous microorganisms was investigated by treating leafy greens (lettuce, sesame leaves).
Methods: The sample was treated with a pilot-scale IPL device. To confirm the microbial inactivation effect according to voltage and treatment time, the frequency (2 Hz), pulse duration (50 ms), and the distance between the lamp and sample (9 cm) were fixed, and the voltage (800-2000 V) and treatment time (1, 2, 4 min) were controlled. The treated samples were cultured on a plate count agar for 48 hours at 37°C and Dichloran Rose Bengal Chloramphenicol agar for 96 hours at 25℃. The temperature and moisture content were measured to determine whether the IPL treatment affected the physical properties.
Results: At the maximum IPL condition (total fluence of 10.5 J/cm^2), the lettuce and sesame leaves exhibited 1.48±0.29 log and 1.55±0.31 log CFU/g reductions in the number of general bacteria, 1.37±0.73 and 1.51±0.57 log reductions in the number of yeast and mold, respectively. The results showed that IPL effectiveness increased with increasing voltage and treatment time. Lettuce, which has a more curved surface, showed lower microbial reduction compared to sesame leaves. There were no significant differences in the temperature of the product surface and the moisture content after IPL treatments (p>0.05*).*
Significance: It is suggested that the IPL treatment may be suitable for controlling indigenous microorganisms on foods, which consumed without heat treatment, like leafy greens with thin and large surface area.

P1-65 Optimization of Pulsed UV-Light Application on the Inactivation of *Listeria monocytogenes* in Cold-Smoked Salmon

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Introduction: *Listeria monocytogenes* is a significant food safety concern, and related outbreaks are often associated with ready-to-eat (RTE) cold-smoked salmon (CSS), which is often consumed without any heat treatment. Therefore, the safety of consuming CSS has been under scrutiny due to lack of listericidal steps in cold-smoking process. A non-thermal technology, pulsed UV light (PUL) treatment, is considered effective in inactivating foodborne pathogens in food systems. However, extensive research is needed to improve PUL processing effects on smoked salmon products.
Purpose: This study aimed to determine the antibacterial efficacy of PUL treatment against *L. monocytogenes* on cold-smoked salmon.
Methods: Pre-sliced, vacuum-packed CSS samples were purchased from a local store in Huntsville, Alabama. The sample was divided into fifteen fillets. Each fillet was inoculated with three different *L. monocytogenes* serovars inoculum:1/2a (ATCC-19111), 1/2b (ATCC-BAA2658), and 4b (ATCC-19115). The inoculated CSS fillets were treated with the PUL system using three voltage levels of 0, 2, and 3 kV and at a PUL energy of 300 J/Pulse, with two energy levels

of 120 and 160 pulses. All the samples were analyzed in triplicates, and statistical analysis was conducted at a 5 % significance level.

Results: The samples treated with PUL at 3 kV and 160 pulses had the most significant *L. monocytogenes* reduction, 1.15 (± 0.07) CFU/g. In comparison, samples treated at 2 kV with 160 pulses showed a log reduction of 0.55 (± 0.07) CFU/g. The treated groups showed a microbial reduction compared to the control (8.35 (± 0.2) CFU/g). No significant difference was observed between 120 and 160 pulses when treated at 2 kV. Increasing the number of pulses and voltage resulted in a significant *L. monocytogenes* reduction in CSS samples.

Significance: The results showed that PUL technology effectively inactivated LM in cold-smoked salmon and has potential application in the CSS industry.

P1-66 Effects of Intense Pulsed Light on Inactivation of *Salmonella* Typhimurium and Quality Characteristics of Pecan Halves

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Introduction: Tree nuts have been linked to foodborne outbreaks and recalls in the U.S. While thermal decontamination methods can inactivate pathogens, they come with challenges of varying severity on the product quality.

Purpose: This study aimed to evaluate the effects of nonthermal intense pulsed light (IPL) on *Salmonella* Typhimurium (ATCC 14028) inactivation and the quality of pecan halves.

Methods: *Salmonella* inoculated pecan halves were treated at distances of 8.28, 10.82, or 13.36 cm for 10, 20, or 40 s using a Z-1000 sterilizing system. The pulsed light system generates 1.27 J/cm^2 pulse of radiant energy at 1.90 cm below the lamp surface, emitting a light spectrum between 200 to 1100 nm. *Salmonella* were counted on tryptic soy agar supplemented with 50 µg/mL nalidixic acid. Uninoculated pecans treated with IPL were evaluated for quality. Log reduction (n=18) and quality data (n=3) were statistically analyzed using SAS (ANOVA and Tukey's test, $p \leq 0.05$).

Results: A significant effect of time and distance ($p \leq 0.05$) on bacterial populations was observed. For instance, IPL treatment for 10, 20, and 40 s at 8.28 cm reduced *Salmonella* population by 1.58±0.10, 3.13±0.19, and 4.55±0.12 log CFU/pecan, respectively. At 10.82 cm, reductions were 1.31 ± 0.09, 2.84 ± 0.19, and 3.94 ± 0.18 log CFU/pecan for the corresponding time intervals. Similarly, at 13.36 cm, reductions were 1.16 ± 0.10, 2.11 ± 0.13, and 3.43 ± 0.14 log CFU/pecan, for 10, 20, and 40 s, respectively. No significant difference in color, texture, water activity, moisture content, and peroxide value was observed between control and IPL-treated samples. However, increase in treatment time led to a decrease in the mass of total monosaccharides.

Significance: This study demonstrates that IPL treatment holds promise as a decontamination method for pecan halves, suggesting potential industrial application.

P1-67 Efficacy of Intense Pulsed Light and Cold Plasma Technologies for *Escherichia coli* Inactivation on Pecan Halves

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Introduction: During processing, tree nuts can be contaminated with foodborne pathogens that demand the need for using novel nonthermal techniques for enhancing microbial safety.

Purpose: This study evaluated the efficacy of intense pulsed light (IPL) and cold plasma techniques to inactivate *E. coli* on pecan halves.

Methods: Pecan halves were spot inoculated with ~ 6 log CFU/pecan *Escherichia coli* (ATCC 8739) and then treated with IPL using a Z-1000 modular sterilization system (1.27J/cm^2 pulse of energy) and cold plasma. Cold plasma was generated using the Enercon Blown-arc Plasma system at 900 Hz in atmospheric conditions. Treatment times of 12 and 15 s, and distances of 4.5, 7, and 9.5 cm were applied for both IPL and cold plasma. Samples were then homogenized, serially diluted, plated, incubated at 37 °C, and colonies were counted after 24 h. Each value was the mean of nine independent experiments performed in triplicates (n=18). Data were compared by ANOVA and Tukey's test using SAS to determine significant difference among means ($p \leq 0.05$).

Results: Log reductions obtained by IPL varied from 0.74 to 2.63 CFU/pecan. The highest reduction (2.63 log CFU/pecan) was achieved when samples were treated at 4.5 cm for 15 s, however, it resulted in pecan charring. The optimal reduction without charring was 2.06 log CFU/pecan at 7 cm and 12 s. IPL treatment time and distance had a significant effect on log reduction ($p \leq 0.05$). Cold plasma resulted in a reduction ranging between 0.59-1.0 log CFU/pecan without charring. Cold plasma treatment time and distance had no significant effect on log reduction. Results showed IPL to be more effective than cold plasma.

Significance: This study demonstrates that IPL and cold plasma have the potential to effectively increase the microbiological safety of pecan halves and could be a promising technology for nut safety.

P1-68 Application of Subcritical Water Extraction (SWE) Technology for the Extraction of Bioactive Compounds from Ginger (*Zingiber officinale*)

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Introduction: Subcritical water extraction (SWE) is a novel technology that is well known due to its high food safety and beneficial environmental effects, in which it efficiently extracts bioactive compounds from plants using water as a solvent. In this study, we applied extraction models to predict the extraction yields of 6-gingerol, 6-shogaol, and zingerone which are bioactive compounds contained in ginger.

Purpose: This study was conducted to find optimum conditions for extracting bioactive compounds from ginger by using SWE and suitable extraction kinetic models.

Methods: Uniform ginger particles were selected and used in the experiment. SWE was performed using an accelerated solvent extractor (ASE 350, DIONEX Co.) with purified water. In every experiment, 1g of dried ginger sample and 1g of diatomaceous earth were placed in a stainless-steel extraction cell, and the extraction was carried out at 200°C for 5, 10, 20, 25 and 30 min. Quantitative analysis was performed using high-performance liquid chromatography with a C18 column. Adapting the model proposed by Peleg, the extraction yield according to the extraction time was substituted into the equation of this model and the extraction process was analyzed.

Results: The equilibrium extraction yields (mg/g sample) of 6-gingerol, 6-shogaol, and zingerone at 200°C were 0.957, 2.106, and 2.476 respectively, and the process of extraction were fitted well using the Peleg model with the R^2 values of 0.982, 0.995, and 0.951 respectively. The results also showed that the yield of zingerone increased, but the yield of 6-gingerol decreased as the extraction time increased. The yield of 6-shogaol has no significant difference with the extraction time.

Significance: It was confirmed that the proposed model described the extraction kinetics with reasonable accuracy and the results could be used as basic data for industrial-scale applications of subcritical water extraction.

P1-69 Increasing Food Manufacturing Capacity for Local Produce through Rural Shared-Use Manufacturing Space

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Introduction: As locally-sourced food is trending, food entrepreneurs are exploring local food product development, but understanding manufacturing practices, food safety, and regulatory requirements is complex. Thus, the Share Grounds, value-added food project team, at the University of Arkansas Extension developed the Veggie to Value (V2V) program to work with local food entrepreneurs from concept through product development.

Purpose: Pilot a 3-month intensive food manufacturing boot camp using locally sourced produce.

Methods: Clients were recruited through an application requesting information about food manufacturing capacity; three clients were chosen from a pool of ten applicants. From May through September 2023, clients participated in a series of one-on-one sessions to learn about the program requirements, food manufacturing, and food safety regulations. The clients chose seasonally available produce from Barnhill Orchards in Lonoke, Arkansas and developed a recipe. The clients created product prototypes modifying recipe concepts. Once the recipe was standardized and any process authority approvals were obtained, clients completed production runs, record keeping, quality testing, and labeling. Products were featured at the Veggie to Value Workshop and Showcase, September 2023.

Results: All three V2V clients successfully completed the program and product development. All clients touted the program for being comprehensive and thorough in design, but two clients recommended lengthening the program from 3 months to one year to give clients more time to learn and practice concepts. Two clients were establishing homesteading operations, and one client was interested in exploring independent manufacturing. All clients plan to continue with food manufacturing businesses.

Significance: Locally made food products are growing in popularity especially as states including Arkansas pass Food Freedom legislation for home-based food manufacturing. These laws have the potential to increase regionally available food supplies, but reintroducing the public to food processing and preservation will need adequate outreach, training, and technical assistance.

P1-70 Survival of *Listeria monocytogenes*, Yeast and Mold on Fresh Pears with Different Storage Temperatures

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Introduction: As pears are the second most produced and consumed pome fruit worldwide, *Listeria monocytogenes* a significant foodborne pathogen that can survive on pears for an extended duration. However, there is sparse information about its fate on fresh pears under various storage temperatures.

Purpose: To comprehensively examine the survival of *L. monocytogenes* on fresh pears of the selected varieties at recommended and abusive storage temperatures over a 20-week storage period.

Methods: Pears of the selected varieties were inoculated with ~6.5 log CFU/pear *L. monocytogenes* and subjected to simulated storage at 0°C, 10°C, and room temperature (RT) for up to 20 weeks. The enumeration of *L. monocytogenes*, as well as yeasts and molds were conducted at regular intervals and quantified throughout the storage period.

Results: No significant initial decline in *L. monocytogenes* populations was observed within the first 24 hours after inoculation, regardless of pear variety. In a short-term storage (14 days), a gradual decline in population occurred, with the most pronounced reduction at room temperature storage ranging from 2.24 to 2.41 log. However, this decline was not statistically significant compared to 0°C (1.40-2.09 log) and 10°C (1.39-2.19 log). The initial yeast and mold levels were 5-6 log CFU/pear and remained stable at 0°C but exhibited slight increases at 10°C and RT. In 20 weeks of cold storage, *L. monocytogenes* in pears, starting from ~6.5log CFU/pear, steadily declined, irrespective of varieties, however, a residual level of more than 3 log of *L. monocytogenes* remained after 20 weeks.

Significance: This study highlights the resilience of *L. monocytogenes* in fresh pears during storage, underscoring the importance of implementing robust food safety measures in the pear supply chain.

P1-71 Detection of *Listeria* spp. using A Microtally® Mitt and Pre-Moist Sticksponge™ for the Environmental Sampling of Three Different Inoculated Surfaces

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Introduction: Processing plants' major concern is *Listeria monocytogenes*. Environmental monitoring programs use *Listeria* spp. for surveillance. Environmental sample collection must be efficient, effective, and practical. The MircroTally® Mitt is a simple food sampling tool, however it has not been tested for environmental samples.

Purpose: To evaluate the use of MicroTally® Mitt (dry) and pre-moistened StickSponge™ to detect *Listeria* spp. in environmental samples using three different inoculated surfaces (Stainless Steel sheet, Teflon Cutting Board, Stainless steel drains).

Methods: Environmental swab StickSponge™ samples (n=216) and MicroTally® Mitt samples (n=216) were collected from three different inoculated surfaces. Surfaces were inoculated with a cocktail of *L. monocytogenes, L. innocua,* and *L. welshimeri* at 4 different concentrations (1, 2, 3, and 4 log CFU/cm^2). The presence of *L. monocytogenes and Listeria spp.* were determined using a commercial PCR system and direct plating on MOX for result confirmation.

Results*:* There was a statistically significant difference in recovery between swabs and mitts (p<0.05). A total of 197 (91%) positive samples for the swabs with 61 (85%) positive samples on the stainless-steel sheet, 72 (100%) on Teflon, and 64 (89%) positive samples on the drains. The mitt with 54 (25%) total positive samples showed that there was less recovery. Only 9 (13%) positive samples on stainless steel sheet, on the Teflon 16 (22%) were positive. The drains had a better outcome with 29 (40%) positive samples.

Significance: The mitt, a commercial sample technique, may not have recovered because it was not pre-moistened. This study will next evaluate pre-moistened mitt recovery.

P1-72 Validation of Cranberry Muffin Baking Process to Control *Salmonella* Contamination

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Introduction: *Salmonella* has been linked with foodborne illnesses resulting from consumption of contaminated fresh fruits. When potentially contaminated fruits are used in preparing batter for baked food, it becomes even more critical to ensure the safety of finished baked products.

Purpose: Validate the effectiveness of a simulated cranberry muffin baking process as an effective pathogen kill step for *Salmonella,* when cranberries were used as a source of contamination.

Methods: Fresh cranberries (160g) were mist inoculated with a 4-serovar cocktail of *Salmonella* to achieve 8.68 ± 0.03 log CFU/g. The stand mixer was used to mix 445 g of white sugar, Premix-1, egg whites, canola oil, buttermilk, water, and crushed oranges. Later, inoculated cranberries and unbleached wheat flour were added to prepare the muffin batter. A 135 g of the prepared batter was weighed and placed in the muffin liners before freezing the muffins at -20°C for 12 hours by sprinkling 1 g of Demerara sugar. After 12 hours, the muffins were baked at 300°F for 45 minutes followed by 15 minutes of ambient air cooling. Muffin samples were drawn at 0, 9, 18, 27, 36, 45, and 60-min for *Salmonella*, a_w, and pH analysis. *Salmonella* in pre and post baked

Poster

samples were enumerated on injury recovery media (Brain heart infusion agar over-layered with Xylose Lysine Deoxycholate agar (XLD).

Results: The *Salmonella* populations decreased from 7.86±0.25 log CFU/g to 3.26±1.05 log CFU/g, resulting in a 4.60 log reduction in *Salmonella* populations. The a_w significantly decreased during the baking process whereas pH varied throughout the process.

Significance: This study validates that baking cranberry muffins at 300°F for at least 45 minutes will result in a 4.60 log (CFU/g) reduction in *Salmonella* populations.

P1-73 Hurdle Approach to Simulate Corn Wet Milling Inactivation of Undesirable Microorganisms: A Pilot Scale Microbial Challenge Study Using *Salmonella* Surrogate *Enterococcus faecium*

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❖ ***Developing Scientist Entrant***

Introduction: Corn wet milling processing conditions reduce undesirable microorganisms, like pathogens. Finished products are historically safe, with intrinsic properties that also inhibit microbial growth. Processors could use quantified levels of reduction and growth inhibition from these processes for developing updated food safety plans.

Purpose: Quantify microbial reduction of *Salmonella* surrogate *Enterococcus faecium* at multiple industry-relevant processing conditions, to demonstrate very low inherent microbial risk.

Methods: Steeping was tested at pilot scale with low (750 ppm SO_2, 20 hours, 43.3°C), medium (1,500 ppm SO_2, 30 hours, 48.9°C), and high (2,200 ppm SO_2, 40 hours, 53.3°C) treatment conditions. Peroxide was tested on bench scale with a factorial design [three pHs (3.5, 4.0, 4.5), H_2O_2 concentrations (0.05%, 0.10%, 0.15% w/w), and temperatures (32°C, 38°C, 46°C)] for 3 and 6 hours. Flash drying was tested at pilot scale at four different temperatures (149°C, 177°C, 204°C, 232°C) with two different inoculation methods. An additional study quantified microbial survival during storage from the starch sampled from different drying temperatures from weeks 0 to 8.

Results: Steeping had a reduction ranging from 3.4 to >6.5 log CFU/g, from low and high treatment levels respectively. The peroxide step had a reduction range from no change to >6 log CFU/g at the highest intensity (0.15%w/w H_2O_2). Flash drying had a reduction range from 2.3 to 3.0 log CFU/g, from low to high temperatures, respectively. Using these unit operations cumulatively leads to an overall reduction of 5.7 log CFU/g reduction if only low-intensity treatments are selected to >15 log CFU/g if only high-intensity treatments are selected. There was no meaningful change <1 log CFU/g in *E. faecium* counts throughout the survival study.

Significance: This hurdle approach study shows that existing corn wet milling conditions are effective for *Salmonella* surrogate reduction through processing steps, into finished starch, demonstrating very low microbial risk in corn wet milling and finished products.

P1-74 Impedance Biosensor for Accurate Detection of *Salmonella* in Raw Chicken Products

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella,* a leading cause of foodborne illnesses *in the U.S and globally,* imposes an annual economic burden of $3.7 billion for healthcare, reduced productivity, and fatalities. Despite national goals for improvement, the infection rates have stagnated for three decades, necessitating faster detection methods to combat this persistent public health challenge.

Purpose: The objective of this study is to validate impedance-based microfluidic biosensor for concentrating, trapping, and rapid detection of *Salmonella* Typhimurium in raw chicken samples.

Methods: The device consists of three key regions (1) The focusing region concentrates *Salmonella* cells at the microchannel's center to a detectable level, directing them toward the detection zone. (2) The trapping region stops *Salmonella* on the top of detection electrode, facilitating antibody-antigen binding. (3) The detection region features two sets of interdigitated electrodes (IDE) arrays. One set is coated with a specific crosslinked antibody, while the other serves as a negative control. Raw chicken samples spiked with *Salmonella* loaded into the sample inlet travels from the focusing to the detection region. The binding of the *Salmonella* antigen to its antibody induces impedance changes, indicating the presence or absence of *Salmonella*.

Results: The biosensor was able to detect *Salmonella* with high selectivity, specificity, and limit of detection of 1-2 cells/ml in < 1 hour. Notably, it differentiated low concentration of *Salmonella* in the presence of high concentration of dead *Salmonella* (1395 cells/mL), and non-specific binding of *Listeria* (1426 cells/mL) and *E. coli O157:H7* (1820 cells/mL).

Significance: This project aims to develop a portable, user-friendly, and cost-effective biosensor for rapid *Salmonella* detection in poultry products, empowering poultry companies and health stakeholders o swiftly implement interventions while improving data environments and analytics to enhance food safety. Furthermore, the device's adaptability allows for the detection of other foodborne pathogens in beef, dairy, green leaf, and water products.

P1-75 Detection of *Salmonella enterica* and *Escherichia coli* on Vegetables Sold Utilizing Two Different Selling Methods in Fresh Food Markets in Battambang Province, Cambodia

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Introduction: Display methods for fresh vegetables in Cambodian fresh food markets may influence bacterial contamination rates of vegetables.

Purpose*:* To determine the prevalence of *E. coli* and *Salmonella* on lettuce and cucumbers displayed for sale either on the ground or on tables in Cambodian fresh food markets.

Methods*:* A total of 80 vegetable samples were collected from 40 vendors displaying vegetables for sale (specifically cucumbers and lettuce) either on the ground or on tables, in fresh food markets in Battambang Province during both dry and rainy seasons. *E. coli* and *Salmonella* were cultured from samples utilizing traditional microbiological methods and putative colonies confirmed to the species level by PCR. Data were analyzed using generalized linear mixed models.

Results: For *E. coli,* a significant interaction was apparent between display method and season (p=0.04), as well as between display method and vegetable type (p=0.03). Whereby, seasonal and vegetable type differences in *E. coli* prevalence were specific to display method (on ground vs. on table). Cucumber samples displayed on tables had a significantly lower prevalence of *E. coli* (estimates [95% CI]: 2.1 [1.1, 29.1] %) as compared to cucumbers displayed on the ground (56.9 [27.9, 81.8] %) and lettuce samples displayed using either method. Furthermore, during the rainy season, estimated *E. coli* prevalence was significantly lower on samples being displayed on tables (10 [0.02, 29.4] %) as compared to samples displayed on the ground (55.1 [28.9, 78.6] %) and samples displayed using either method during the dry season. Only 3 samples were contaminated with *Salmonella,* all corresponding to the on the ground display method. Low detection rates for *Salmonella* prevented further statistical analysis.

Significance: Our findings support the use of tables to display vegetables for sale in Cambodian fresh markets to decrease *E. coli* contamination of vegetables, particularly during the rainy season when overall contamination rates are higher.

P1-76 Prevalence of *Escherichia coli* and *Salmonella enterica* in the Vegetable Value Chain in the Province of Siem Reap, Cambodia

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Introduction: Poor hygienic practices within the vegetable value chain may contribute to an increased risk of foodborne infectious diseases in Cambodia.

Purpose: To assess the contamination rates of *E. coli* and *Salmonella* in commonly consumed raw vegetables, namely lettuce, tomato, and cucumber, as well as environmental samples taken from both food contact (FC) and non-food contact (NFC) surfaces along the value chain in the province of Siem Reap, Cambodia.

Methods: A total of 704 samples were collected between April and November 2022, comprising 480 vegetable samples and 224 environmental samples. Samples were collected from farms and vendors from distribution centers (DC) and markets. Bacterial culturing methods were performed to identify the presence of *E. coli* and *Salmonella*. Characteristic colonies were isolated and further confirmed using RT-PCR.

Results: The overall prevalence of *Salmonella* was considered low (35/704) with no significant differences observed along the value chain (estimates [95% CI]: Farm 1 [0.3, 5]%, DC 11 [5, 24]%, market 15 [7, 29]%) regardless of season or sample type. *Salmonella* prevalence was found to be higher during the rainy season compared to the dry season (16 [10, 24] % and 2 [0.6, 8]%, respectively). The prevalence of *E. coli* in lettuce and on FC surfaces was significantly higher than in other sample types (lettuce 53 [36, 68]%, cucumber 22 [12, 38]%, FC 53 [37, 68]%, NFC 38 [24, 53]%). Seasonal variations did not show a significant association with *E. coli* detection rates for any of the sample types.

Significance: In this study, no significant differences were observed throughout the various stages of the vegetable value chain for *Salmonella* or *E. coli*. Moreover, although the overall *Salmonella* prevalence was low, results revealed a higher prevalence of *Salmonella* during the rainy season, emphasizing seasonal considerations. *E. coli* exhibited a high prevalence overall, notably on lettuce and food contact surface samples.

P1-77 Identification of Appropriate Concentrations of Ascaroside#18 in the Control of *Salmonella enterica* and Enterohemorrhagic *Escherichia coli* on Alfalfa Seeds and Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: Evidence has substantiated the efficacy of ascaroside#18 treatment in the control of *Salmonella* and EHEC growth in alfalfa seeds/sprouts, as indicated in several prior studies. However, the minimal and optimal concentrations of the treatment have not been determined.

Purpose: This study compared the efficacies of four different concentrations (1 mM, 0.1 mM, 10 mM, and 1 mM) of ascaroside#18 in control *Salmonella* and EHEC (2 strains each) growth on alfalfa seeds/sprouts.

Methods: Sanitized commercial alfalfa seeds were exposed to the four respective concentrations of ascaroside#18 for 30 min before being immersed into each of the four bacterial suspensions (ca. 10^3 log CFU/ml) for 1 h. The seeds were sprouted on 1% water agar with or without the supplementation of an appropriate concentration of ascaroside#18 at 25ºC. Inoculated seeds not treated with ascaroside#18 were sprouted on regular 1% water agar as treatment controls. On days 0, 1, 3, 5, and 7, *Salmonella* and EHEC populations in collected samples were enumerated. The data was analyzed using ANOVA of SAS.

Results: Results of statistical analyses showed that bacterial strain type, treatment concentration, and sprouting time were the main effects (*P*£0.05) influencing *Salmonella* and EHEC populations on seeds/sprouts. *S*. Cubana had a significantly higher population than other tested strains. The minimal inhibitory concentration of ascaroside#18 was 1 mM, and the most effective concentration was 1 mM among the concentrations tested. There was no significant difference in *Salmonella* and EHEC populations recovered from the two types of water agar. The peak pathogen populations were retrieved on day 7 with dramatic increases occurring from day 0 to day 1.

Significance: The study identified the minimal and most effective concentrations of ascaroside#18 treatment among the 4 tested concentrations for control of *Salmonella* and EHEC growth on alfalfa seeds/sprouts. Continuous exposure to ascaroside#18 during sprouting was unnecessary to enhance its inhibitory effect.

P1-78 Validation of White Nectarine Drying Process to Control *Salmonella* and Shiga Toxin-Producing *Escherichia coli*

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Introduction: Dried fruits are a popular healthy snack that provides essential vitamins, minerals, fiber and antioxidants. However, the microbial safety of dried fruits has attracted increasing attention in recent years due to recalls associated with potential bacterial pathogen contamination.

Purpose: To validate sliced white nectarine drying process to control *Salmonella* and Shiga toxin producing *Escherichia coli*.

Methods: Two independent lab-simulated sliced white nectarine drying processes were conducted to study the survivability of *Salmonella* and STEC. A commercial fruit slicer was used to cut the nectarines into ~1 cm thick slices. Two batches of sliced nectarines (~15 slices) spread in two different containers were mist inoculated with 4-serovar *Salmonella* and 5-strain STEC cocktail, respectively. After inoculating each side of the sliced nectarines, a 30-minute stabilization period was provided, allowing the bacteria to attach to the fruit surface. After the inoculation and stabilization period, the nectarines slices were moved onto a stainless-steel wire oven rack and nectarines were dried in an oven for 10 hours at 75°C (167°F). A 6-channel data logger was used to monitor the internal temperature of the sliced nectarines with one channel measuring the oven air temperature. The samples were analyzed for surviving respective bacterial populations, water activity, pH, and the experiments were replicated three times.

Results: After the mist-inoculation, white nectarine slices retained 10.28±0.24 and 9.60±0.21 log CFU/g of *Salmonella* and STEC population, respectively. However, at the end of drying, the populations of *Salmonella* and STEC decreased by 9.74 and 9.06 log CFU/g, respectively.

Significance: This is the first scientific study demonstrating the survivability of *Salmonella* and STEC on sliced white nectarines during the drying process. The results will enable the fruit drying industry to optimize the oven drying process thus implementing an effective preventive control assuring food safety.

P1-79 Microbial Transfer and Cross-Contamination of *Escherichia coli* in a Wheat Milling Facility

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❖ ***Developing Scientist Entrant***

Introduction: Microbial contaminants entering the mill with incoming wheat kernels and the accumulation of grain or flour residues within milling equipment present significant risks for cross-contamination during processing. This potential transfer of microorganisms from equipment to food raises serious food safety concerns, often associated with pathogen cross-contamination in food processing environments, a leading cause behind food recalls.

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Purpose: To quantify the distribution and transfer of *Escherichia coli* contamination from wheat to mill equipment and wheat flour fractions during milling processing.

Methods: Soft Red Winter (SRW) wheat was inoculated with a five-strain cocktail of non-pathogenic *Escherichia coli* (ATCC 1427, 1428, 1429, 1430, and 1431) and processed in a pilot scale milling facility. To determine microbial load, milling fraction samples were collected during the milling of inoculated wheat (at 20, 40, and 60 min) and non-inoculated wheat (at 30, 60, 90, 120 min). Equipment surfaces were swabbed after processing inoculated wheat, and after processing non-inoculated wheat. Additionally, fractions and equipment samples were collected after implementing a cleaning intervention.

Results: When milling wheat inoculated with *E. coli* (~4.5 log CFU/g), high microbial transfer to non-flour milling fractions (~3.6 log CFU/g) was observed, while significant contamination levels were observed in straight-grade flour (~2.6 log CFU/g). Contamination was also recovered from milling equipment surfaces, which showed quantifiable microbial counts (up to 2.3 log CFU/100 cm^2). The subsequent processing of clean wheat (non-inoculated wheat), plus the implementation of a cleaning intervention, effectively reduced microbial contamination in flour fractions (<1 log CFU/g) and equipment (<1 log CFU/100 cm^2).

Significance: This study shows that cross-contamination in wheat milling processing can easily occur. Importantly, it demonstrates that contaminants can be flushed through the system using grains that are free of microbial contamination and by implementing simple cleaning interventions.

P1-80 Antimicrobial Resistance of *E. coli*-Dominated Multi-Species Biofilms on Sub-MIC of Grapefruit Seed Extract

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Introduction: Multi-species biofilms are known to have more antimicrobial resistance than the mono-species biofilms, therefore understanding the mechanisms underlying multi-species biofilm formation is crucial for ensuring food safety.

Purpose: This study aimed to investigate the multi-species biofilm formation of *Escherichia* coli, *Salmonella* Typhimurium and *Listeria monocytogenes* and to evaluate antimicrobial resistance on sub-minimum inhibitory concentration (MIC) of grapefruit seed extract (GSE) among various biofilms was investigated.

Methods: This study investigated the formation of mono-species, dual-species, and multi-species biofilms as well as antimicrobial resistance and counterintuitive effect in response to GSE at various concentrations including MIC and sub-MIC (1/2 MIC and 1/4 MIC). In addition, for the evaluation of mechanical analysis of antimicrobial resistance, the motility test, biofilm formation analysis using the crystal violet (CV) assay, field emission-scanning electron microscopes (FE-SEM), and genes related to the quorum sensing (QS), stress response, flagella and curlin subunit were investigated.

Result: The results of the swimming and swarming motility tests revealed a counterintuitive effect, with increased motility observed in the sub-MIC concentration of GSE. The CV assay demonstrated increased biofilm formation in multi-species biofilms, highlighting the synergistic effect of *E. coli*, *S.* Typhimurium, and *L. monocytogenes*. At the MIC concentration of GSE, FE-SEM revealed cell damage and destruction of cell morphology, while sub-MIC concentrations increased biofilm formation and architectural complexity. Multi-species biofilms demonstrated greater biofilm-forming ability and antimicrobial resistance than mono-species biofilms, indicating synergistic interactions and enhanced resilience.

Significance: Our findings revealed a substantial increase in biofilm formation as bacterial strains were added. In addition, this study also highlighted an enhanced adaptive response in multi-species biofilms compared to mono-species, suggesting potential synergistic effects and increased resilience within mixed populations.

P1-81 Monitoring the Effect of Bioprotective Cultures on the Fate of *Escherichia coli* during Storage of Fresh-Cut White Cabbage

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Introduction: Fresh□cut salads have gained worldwide attention due to consumers demand for a healthier diet. However, numerous foodborne disease outbreaks associated with fresh produce or minimally processed salads have been reported worldwide.

Purpose: This work studied the effect of *Lactiplantibacillus pentosus* FMCC-B281 free cells and its supernatant on the fate of *Escherichia coli* during storage of fresh-cut white cabbage.

Methods: White cabbage (WC) was thoroughly washed with water to remove any organic material and was dried for 30 min in a laminar flow cabinet before use. *L. pentosus* and *E. coli* were grown at 37°C for 24h in MRS and BHI broths respectively and the cultures were centrifuged to collect the supernatant and the pellet (free cells). Free cells (F, as ~5 log CFU/g), supernatant (S) and control (C, broth) were used to spray the leaves that had been previously contaminated (sprayed) with free cells of the pathogen (4 log CFU/g). Then, the salads were packaged under modified atmosphere packaging ($10\%CO_2/10\%O_2/80\%N_2$) and stored at 4 and 10°C until spoilage. During storage, microbiological and pH analysis were performed.

Results: During cold storage, pathogen population increased by 3 log CFU/g at control samples, whereas at S and F-samples pathogen growth was maintained at lower levels (ca. 5.5 log CFU/g). At 10°C, pathogen increased rapidly and achieved higher populations (8.5 log CFU/g). Spoilage was intense and shelf-life was shorter at control, compared with F and S-samples, where shelf-life was elongated by 3 days. pH values were similar during storage at both temperatures.

Significance: The applied treatments can elongate the shelf-life of the fresh-cut salads and provide a mild antimicrobial action against foodborne pathogens.

P1-82 Modernizing HACCP Systems: A Structured Data Approach

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Introduction: Hazard Analysis and Critical Control Points (HACCP) is the systematic preventive approach to food safety, applied throughout the food chain by all stakeholders. Many Food and Beverage (F&B) companies manage their HACCP studies using documents or spreadsheets. Such systems can lack flexibility and are often cumbersome to maintain and revise, especially with regards to risk change management. Data on hazards and their controls is "locked" in these documents preventing data and information being easily reused across HACCP studies.

Purpose: In this presentation, we provide some of our understanding, gained by working with leading F&B companies, on the promises of a data-centric approach to digital transformation of HACCP.

Methods: We'll introduce the concept of a structured data approach, discuss the benefits, and illustrate how a structured data approach provides the foundation for a fully digital pipeline of HACCP management.

Results: Such a pipeline places HACCP at the center, as the control tower of the food safety management system, integrating data-driven inputs such as early warning, material intrinsic risk, supplier performance and validated control measures with outputs such as manufacturing process control as monitoring/verification activities.

Significance: We will illustrate the benefits of a digitalized structured data approach to HACCP; an approach that provides agility, reduces complexity, helps eliminate errors/misalignment across studies and enables data flows across connected systems.

P1-83 Model Food Safety Plan for Dietary Supplements

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Introduction: No Food Safety Model Plans have been developed for Dietary Supplements; this has been identified as a need by industry professionals.
Purpose: The purpose of this project was to create a model Food Safety Plan (following FSPCA teaching models) for a dietary supplement operation and present both voluntary and compulsory regulations to this type of product.
Methods: The methods used to create the model Food Safety Plan (FSP) follow the procedures created by the Alliance to develop model plans, FSPCA Guidelines for Industry-specific FSP Teaching Examples for FSPCA Human Food Training. The policy requires that the model plans must be based on a realistic product, must use the FSPCA FSP teaching example format, and must include all preliminary steps (such as company and product description, including food safety characteristics; ingredients; allergens; packaging used; intended consumers and use; shelf life; labeling instructions; storage and distribution), flow diagram, and process narrative. Lastly, it must have at least one preventive control. This FSP includes all four types of preventive controls in order to support different product delivery formats. This project is specific to a supplement for joint health.
Results: The hazard analysis process within the FSP pinpoints steps in production where a known or reasonably foreseeable hazard could occur. These could be inherent in the dietary supplement being produced or part of the production process. Using a risk-based approach, preventive controls were assigned to mitigate or eliminate the hazards most likely to occur. Identifying and applying relevant controls ensures compliance with both foods and dietary supplement regulations and reduces the risk of food safety issues.
Significance: Availability of these Food Safety Plan Model is of significance for small and medium dietary supplement companies to support the implementation and ongoing maintenance of FSP in their facilities. Staff assigned to the task of food safety implementation may not have the technical knowledge required to create a robust plan.

P1-84 Evaluation of HACCP in Food Manufacturing Companies in the Middle Eastern Region

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Introduction: The way that food produced and distributed has undergone fundamental changes in recent decades particularly in Dubai and the Middle Eastern region. The food safety area has become more complex, driven by widespread changes in methods of food production and processing, coupled with rapid increases in global food trade and increased tourism. Today consumers are demanding more consequential information on food safety and quality. To meet this demand, some companies are engaging food safety management system implementation and third-party certifications to provide greater assurance that their products meet quality and safety requirements.
Purpose: The overall goal for this project was to evaluate the level of implementation and operation of hazard analysis critical control points (HACCP) and PRPs (Prerequisite Programme) in food manufacturing companies in the Dubai Emirates, based on the codex protocol of 12 logical steps and codex GHP (Good Hygiene Practices).
Methods: Both qualitative and quantitative analysis techniques of in-depth interviews, observations, and review of document used in this study. This is to complement each other. The triangulation method used in this research was to look at the problems from different angles. Five cluster random samples were collected from the sampling frame of 112 food-manufacturing companies of Dubai Municipality Food Safety Department listing.
Results: Research identified lower compliance rates of GHPs which compromise 37.4% for the sampled factories and lower compliance rate of codex twelve logical steps implementation which compromise 31.8%.
Significance: The outcomes of this study may have national and international implications for the enhancement of HACCP system implementation. This research was also particularly timely, in light of the falling food safety standards in food manufacturing in the Dubai Emirates.

P1-85 Implementing an Online Complaint Surveillance System in Tennessee

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Introduction: Complaint-based surveillance is used in public health to identify possible foodborne outbreaks. The Tennessee Department of Health's (TDH) traditional method of capturing complaints relies on complaint receipt via telephone and processing within local health departments (LHDs). Alone, traditional complaint-based surveillance limits consumer reporting options and increases time spent by LHD interviewing complainants. Using an online complaint system is an opportunity for consumers to report their complaints easily, anytime, which may increase complaints received and reduce complaint intake time.
Purpose: To improve complaint-based surveillance in Tennessee.
Methods: Tennessee's online complaint system was adapted from Tennessee's traditional system and released on TDH's website in July 2023. Standard operating procedures were developed for triaging complaints based on relevant health and exposure information, including the incubation period, symptomology, healthcare visits, and number of persons ill. This triage strategy quarantines complaints with missing or confounding information and ensures rapid notification of complaints to LHDs. Quarantined complaints are routinely compared to other complaints in the system in case there are other similar exposures.
Results: From July 2023-March 2024, 152 complaints were reported to the traditional system (16.9 monthly average), while 277 were reported to the online system (30.8 monthly average). The traditional system identified 4 (2.6%) outbreaks in that time, while the online system identified 10 (3.6%). Of those complaints, 37.9% (105) were quarantined.
Significance: The introduction of an online complaint surveillance system in Tennessee has increased complaint reporting, which has led to the identification of additional outbreaks. The triage system reduces time burden on LHDs while ensuring implicated food establishments are appropriately investigated.

P1-86 Surveillance of Pathogenic Bacteria on a Food Matrix Using Machine Learning-Enabled Paper Chromogenic Arrays

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Introduction: Global food systems can benefit significantly from continuous monitoring of microbial food safety, a task for which tedious operations, destructive sampling, and the inability to monitor multiple pathogens remain challenging.
Purpose: This study aimed to construct and use a nondestructive and nonculture-based paper chromogenic sensor array-machine learning (PCA-ML) system for simultaneously and continuously detecting multiple foodborne pathogens at low concentrations in foods.
Methods: A paper chromogenic array sensor - machine learning (PCA-ML) methodology, sensing concentrations of volatile organic compounds emitted on a species-specific basis by pathogens, was developed by streamlining dye selection, sensor fabrication, database construction, and machine learning analysis. PCA was fabricated by nine sensitive chemical dyes selected from 25 via principal component analysis. K-fold cross-validation was used to increase ML analysis robustness. The computational efficiency of the PCA-ML system was evaluated by continuous pathogen identification in ground chicken initially contaminated at low bacterial concentrations (~1 to 3 log CFU/g). The PCA-ML system's capability to detect pathogens at loadings as low as 1 log CFU/g is comparable to most microbial testing systems approved by the Association of Official Analytical Chemists and the FDA.
Results: The system enables noncontact, time-dependent, simultaneous monitoring of multiple pathogens (*Listeria monocytogenes*, *Salmonella*, and *E. coli* O157:H7) over time at either 4°C or 25°C. High identification accuracy (>90%) was achieved either at a high level (~3 log CFU/g) of initial pathogen

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contamination or at a low level (~1 log CFU/g).

Significance: The PCA-ML approach has great potential to be used in smart packaging, for extension to other food commodities and bacterial species, and for use in the food supply chain to monitor bacterial contamination continuously and nondestructively. Nondestructive microbial detection using PCA-ML can mitigate risks of foodborne illnesses and alleviate food waste burdens to help improve food system safety, security, and resilience.

P1-87 Interactive Workshop of Best Food Safety Practices on Harvest and Post-Harvest for Elderberry Growers, Processors, and Beginners

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Introduction: The Western blue elderberry (*Sambucus nigra* spp. *cerulea*) is an emerging commercial crop in the US. However, limited guidance exists for growers and producers on managing food safety risks. *Salmonella* and sambunigrin (cyanogenic glycoside) have led to recalls of elderberry products in 2020 and 2022, respectively. As the market expands for blue elderberry, it is important to educate those producing and processing this crop on food safety risk mitigation strategies.

Purpose: Conduct a hands-on workshop to demonstrate food safety practices in harvesting, post-harvest handling, and processing of Western blue elderberry to increase knowledge and promote behavior change for elderberry growers and processors.

Methods: Before the workshop, a drying trial was conducted to determine optimal parameters for dehydrating elderberries using a cabinet dryer, food dehydrator, and freeze dryer. Measurements of Brix, texture, and pH were taken before drying, while water activity, moisture content, and color were measured before and after drying. To assess the increase of knowledge related to key food safety concepts covered in the workshop, a retrospective pre/post learning evaluation and a prospective behavior change assessment was also included.

Results: The measurements for Brix, texture, and pH were 13.7, 26.79, and 3.58 respectively. A substantial reduction (74-80%) in moisture content was observed across all drying methods and the final water activity ranged between 0.29-0.40. Nineteen individuals completed the retrospective pre/post workshop evaluation survey, and the results were analyzed using paired t-test. Across all content areas, there was a significantly higher self-reported level of knowledge after attending the workshop as compared to before ($p < 0.05$).

Significance: The workshop increased knowledge of best food safety practices for elderberry harvesting and processing. Moreover, this work evaluated some key food safety parameters such as the pH of raw elderberries and water activity of dried elderberries.

P1-88 Proficiency Testing of Total Arsenic and Inorganic Arsenic Analysis in Apple Juice and Rice Flour

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Introduction: Arsenic is a harmful element which exists naturally in the environment, soil and groundwater, and can enter the food chain and impose adverse health outcomes to humans. The FDA action level for inorganic arsenic is 10 µg/kg in apple juice and 100 µg/kg in infant rice cereals. Robust testing methodologies and reliable testing results for quantitation of total arsenic (As) and inorganic arsenic (iAs) are important.

Purpose: Evaluate the performance of Food Emergency Response Network (FERN) laboratories for analyzing low levels of As and iAs in apple juice and rice flour through a proficiency testing (PT) program.

Methods: For the apple juice PT, a total of eight blind-coded samples were analyzed by 33 laboratories for As and 13 for iAs. For the rice flour PT, the NIST certified rice flour reference material (1568b) was analyzed by 26 laboratories for As and 15 for iAs. Results reported from the laboratories were statistically analyzed according to ISO 13528 protocol and z-scores were calculated.

Results: For analyzing the apple juice samples spiked with As at 10 and 50 µg/kg, the consensus values of As were 10.25 and 51.78 µg/kg, respectively. For analyzing the apple juice samples spiked with iAs at 5 and 25 µg/kg, the consensus values of iAs were 5.03 and 24.76 µg/kg, respectively. For analyzing the NIST rice flour sample, the consensus values were 290.9 µg/kg for As and 94.3 µg/kg for iAs. The percentage of laboratories with satisfactory performance with z-scores ≤ 2 were 97 % for As and 100% for iAs for the apple juice PT; and 92% for As and 93% for iAs for the rice flour PT.

Significance: The results showed that the majority of the participant laboratories were proficient in detection and quantification of As and iAs, and the methods used were adequate.

P1-89 Microbial Quality and Prevalence of Extended Spectrum Beta-Lactamase Producing Bacteria in Vegetable Salad from Local and Elite Restaurants in Ibadan, Nigeria

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Introduction: Extended-spectrum beta-lactamase (ESBL) producing bacteria are a significant threat to public health due to harbouring of resistance to broad-spectrum antibiotics.

Purpose: In Nigeria, very little information is available about ESBL producing bacteria from food samples.

Methods: Fifty pure cultures were obtained from (30) Ready-to-eat (RTE) vegetable salad purchased from selected local and elite restaurants in Ibadan, Oyo State, Southwest Nigeria. All the isolates were tested against selected panel of antibiotics which include third generation cephalosporin, aminoglycoside, macrolide, glycopeptide and sulfonamide by the disc diffusion method and screened for ESBL production through double disc synergy test. Detection of ESBL genes with specific primers (CTX-M, TEM, and SHV) using Real-Time Polymerase Chain Reaction.

Results: Out of fifty strains, seven were confirmed ESBL producers which were two *Proteus mirabilis*, one each for *Burkholderia cepacia*, *Burkholderia contaminans, Bacillus cereus, Bacillus velezensis* and *Serratia rubidaea*. It was observed that 5/7 potential ESBL producing strains, were positive to bla_{CTX-M}, bla_{TEM}, and bla_{SHV} while no gene was detected in 3/7 strains. Out of the 5 isolates, bla_{TEM} gene was present in two strains; (bla_{TEM} and bla_{CTX-M}) and (bla_{CTX-M} and bla_{SHV}) genes in one strain respectively, while bla_{CTX-M} gene was present in one strain. The antibiotics susceptibility test indicates that the ESBL producing isolates were 100% susceptible to Imipeinem (IPM). The two *Proteus mirabilis* and *Bacillus cereus* were resistant to nine antibiotics while *Serratia rubidaea* exhibited resistant to 2 antibiotics. It could be inferred that 96% of the bacteria isolated in this study were multidrug resistant as they showed resistance to three or more classes of antibiotics among the panel of antibiotics used.

Significance: This study highlights the occurrence of ESBL bacteria in RTE vegetable salad from food sources in Ibadan and the need for food surveillance programs in controlling the spread of antibiotic resistance in local and elite restaurants.

P1-90 Paving the Way for Safe and Sustainable Animal Source Food in Ethiopia: Lessons from Intervention Successes

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❖ ***Developing Scientist Entrant***

Introduction: Ethiopia's reliance on Animal Source Foods (ASF) for vital nutrients is high, despite food safety concerns stemming from production, handling, and processing practices. This study delves into interventions implemented over the past decade to address ASF food safety challenges, scrutinizing

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both their limitations and drivers of success.

Methods: A multi-vocal literature review was carried out to gather information on the food safety intervention. The review combined both published and grey literature with qualitative in-depth interviews of key stakeholders. The methodology included the identification of four peer-reviewed literature reviews and 14 key informant interviews. Additionally, 41 relevant grey literature sources were reviewed through the key informant interviews.

Results*:* Various interventions at different levels were identified, revealing challenges such as inadequate infrastructure (e.g., electricity, roads, clean water, and hygiene supplies), policy deficiencies, a lack of specific financial allocation, weak coordination and collaboration among stakeholders, differing perceptions of food security and food safety due to low awareness levels, and the impact of external factors like COVID-19 and civil unrest. Despite these challenges, the study identifies promising elements promoting intervention success. Renewed government commitment, support from development partners, and the existence of regional legal frameworks offer valuable footholds for improvement.

Significance: Bridging infrastructure gaps, strengthening policy frameworks, and fostering enhanced collaboration among stakeholders are critical steps forward in mitigating food safety risks associated with ASFs in Ethiopia. This nuanced understanding of the intervention landscape paves the way for focused efforts to safeguard the nutritional benefits of ASFs for millions of Ethiopians.

P1-91 Helping Middle Managers Make Sense of and Give Meaning to Food Safety Changes: A Qualitative Systematic Literature Review

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Introduction: The changing food industry landscape demands food companies to be more adaptive in their food safety culture. Middle managers play a vital role in communicating and driving organisational change and innovation by translating and operationalising change through sensemaking.

Purpose*:* This systematic literature review aims to answer the question "through what mechanisms does middle management's sensemaking shape organizational change?"

Methods*:* A systematic literature review was completed using seven databases: Scopus, Web of Science, Science Direct, Taylor & Francis, Wiley, ProQuest, and Emerald Insight. Scholarly, peer-reviewed publications in English language that answered the research question were included. A qualitative thematic analysis using NVivo version 14 was completed to identify themes and core concepts through three iterations.

Results*:* Thirty-eight articles were included in the analysis: 31 case studies, five theoretical studies, and two cross-sectional survey studies. Middle management's sensemaking shapes organisational change through two interconnected mechanisms: (1) social interaction and discourse; (2) emotional balancing and cognitive framing. Creating meaning that is consistently evoked and that corresponds to stakeholders' role identities and socio-cultural codes is pivotal for both mechanisms, through which middle managers actively strategise narratives, "sell ideas", and manage up, across, and down. Conversely, a lack of meaning results in political activities amongst middle managers for securing power. The ability of middle managers to balance employees' emotions and reduce cognitive dissonance in the times of ambiguity is crucial for reducing resistance and getting buy-in. Recent literature identified "prospective sensemaking" as a key enabler for managing risk and understanding business innovation.

Significance: Middle managers connect organizational levels and functions, yet they are often not empowered to drive proactive culture. This study reveals that middle management's sensemaking is key to reduce miscommunications, create meaning, and modulate resistance in, across, and throughout organisations during change. Food industry will learn from other industries to strengthen their adaptability.

P1-92 Digital Transformation and Smart Food Safety Solutions – Emerging Trends in Food Sector

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Introduction: The global covid pandemic that leaves wide ranging and catastrophic effects at normal ways of living, has placed food sector under immense pressure with rapid transformation by replacing manual, outdated and analog processes with modern, fully integrated technology-based solutions. This gives rise to digital innovations and technological advancements, making food sector smarter, safer, and more sustainable.

Purpose: The purpose of this study is to depict and illustrate how the integration and incorporation of smarter food safety technologies immediately reduces the inaccuracies, uncertainties, lead times and enhanced the transparency, authenticity, and traceability turning food safety more automated, customized, and digitalized with real time monitoring and reporting's.

Methods: The present study will further emphasize on paradigm shift that global player from manufacturing, retail and service sector began using robotics, RFID tags for enhanced customer service and brand experience. Similarly global QSR chains have immediately adopted and transformed to technology-based food safety solutions through that they can complete checks in seconds rather than minutes by using high quality IoT sensors, web connected devices, wireless Bluetooth temperature probes and digital checklists.

Significance: Adoption of digital and smart food safety technologies have opened the doors of availability of diverse and rich information for the customers and can deliver high impact results by reducing operational expenses, preventing product loss, minimizing food safety risks, brand protection and to ensure the system with greater innovations, improved quality, and performance, reduce labour costs, lowers energy consumption and above all enhancing the global sustainability development goals.

P1-93 Quantitative Evaluation of Attitudes Associated with Food Safety Culture: A Three-Year Journey of a UK-Based Low-Risk Food and Drink Manufacturer

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Introduction: Food safety culture (FSC) measurement and improvement has become a requirement for all GFSI-certified food safety standards and EU law (852/2004) meaning that many food and drink manufacturing/processing (FDMP) businesses now need to comply. Understanding company FSC is a complex task and is the first step towards compliance. Quantitative assessment of FSC is frequently undertaken within the FDMP sector; bespoke measurement mechanisms may facilitate an in-depth, tailored understanding to address specific company nuances.

Purpose*:* To obtain and compare bespoke, quantitative attitudes toward FSC categories and dimensions in a low-risk FDMP business over a three-year period.

Methods*:* A company-specific, bespoke questionnaire was developed using FSC literature and qualitative research. Online/paper questionnaires were distributed annually to management and operative employees. Likert scale responses (Strongly Agree-Strongly Disagree) were coded and descriptive and comparative statistics (Friedman's test/T-tests) have been used to determine potential changes year-on-year.

Results*:* Overall, sustained improvement was determined in overall FSC attitudes from 2020 to 2022. General attitudes towards food safety improved significantly from 2020 to 2021 ($p<0.001$). Attitudes and attitudinal change towards all FSC dimensions/categories have been determined, for example, a significant increase in positive attitudes towards food safety communication ($p=.004$) was identified between 2020 and 2021; this sustained improvement was determined during 2021-2022. Similarly, there was an increased in perceived understanding of FSC and its importance from 2020-2022.

Significance: Identification of attitudes toward key FSC categories and dimensions can provide an indication of where to target FSC improvement interventions within a company. Analysis of data over a longer period may provide a more accurate indication of strength of FSC in the business.

P1-94 Food Safety Risks Associated with Home Freeze Drying

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❖ ***Developing Scientist Entrant***

Introduction: Home freeze drying is gaining popularity as interests in consuming raw or minimally processed foods and preserving foods have increased among consumers, and purchasing a pilot- or kitchen-scale dryer has become affordable. However, home freeze dried foods are potentially hazardous foods as they can be contaminated during preparation, equipment loading and packaging, and most consumers lack adequate knowledge of the freeze-drying process to adequately control their dryer and determine corrective actions.

Purpose: Provide consumers guidance on the fundamentals of the freeze-drying process and adequate equipment and process controls for microbial food safety using fresh eggs as a case study.

Methods: Fresh eggs were scrambled, inoculated with 10^7 CFU/g each of *Enterococcus faecium* NRRL B-2354 (surrogate for *Salmonella* spp.), *Listeria innocua* (surrogate for *Listeria monocytogenes*), and generic *Escherichia coli* and kept frozen at -20 °C for 24-96 h prior to freeze drying in a pilot-scale unit (Medium Scientific, Harvest Right, Salt Lake City, UT). The samples were dried using either (a) standard or (b) programmed mode. The standard and programmed modes maintained the shelf temperatures at or below 50 and 74 °C, respectively. Under both modes, the ambient temperature inside the dryer were maintained at or below 27 °C throughout the process. Four replications of each mode were conducted, and the following response variables were compared: microbial log reductions, final water activity and moisture content values.

Results: Less than 5-log reductions in all surrogates were observed in freeze dried raw eggs processed using either standard or programmed mode, despite achieving low water activities (below 0.1) and moisture contents (below 10%) suitable for long-term storage.

Significance: These results show that home freeze dried foods are potentially hazardous foods as freeze drying may not provide adequate lethality, despite the dried foods having water activities too low to support microbial growth.

P1-95 Evaluation of a Rapid Method for the Microbial Enumeration of Raw Materials for Ketchup

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Introduction: In the ketchup industry, good manufacturing practices (GMP) requires suitable detection methods at production lines. Chinese standards, such as NY/T 956-2006 (tomato paste) and NF/T 957-2006 (tomato powder), mention aerobic plate count, coliforms, and molds and yeasts are essential tests from the raw material to the end products. The traditional methods need 2–5-day incubation with plate count agar, two-step enumeration/gassing and Rose Bengal Medium, respectively. Alternatively, a rapid method can enumerate in half the time of the GB method.

Purpose*:* To compare the performance of alternative methods with the GB standards for the raw materials of ketchup.

Method*:* Two major raw materials (tomato powder and white sugar) of ketchup were artificially contaminated with *E. coli CMCC(B)44102* for aerobic plate count and coliform items, and *Saccharomyces cerevisiae* and *Aspergillus niger* for yeasts and molds item. *E. coli* was spiked from 10^1 to 10^6 CFU/g, while the two fungi were spiked from 10^1 to 10^3 CFU/g. Neogen® Petrifilm® Rapid Aerobic Count Plates, Coliform Count Plates and Rapid Yeast and Mold Count Plates with GB 4789.2-2022, GB 4789.3-2016 and GB 4789.15-2016 were compared for identification of aerobic plate count, coliform, and yeast and mold, respectively.

Results: For most test items, the mean log difference of rapid method and their relative GB presents less than 0.5 log difference, except Aerobic plate count on tomato powder. Moreover, all the bias of the three items were included with their β-expectation tolerance intervals in accuracy profile study. The relative trueness analysis showed less than a 0.1 bias, with the highest 95% upper limit of 0.38 and the lowest 95% lower limit of -0.45.

Significance: This alternative method offers a rapid screening f or the raw material in the ketchup industry with accurate and repeatable results compared to the traditional agar within 24-48 hours.

P1-96 Evaluation of the Neogen® Environmental Scrub Sampler (ESS) on the Recovery of Microorganisms Present on Food Contact Surfaces in Food Processing Facilities

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Introduction: Food preparation, handling, and processing areas can easily introduce food contamination if improperly cleaned and sanitized. Verification of cleaning procedures by monitoring bacterial load on food contact surfaces is thus important for hygienic food production.

Purpose: This study investigated the overall recovery of microorganisms using the Neogen® Environmental Scrub Sampler (ESS) in the presence of commonly used sanitizing agents in food contact surfaces in processing facilities.

Methods: The recovery of *Escherichia coli* and *Listeria innocua* was evaluated in the presence of sanitizer residues (0.05% quaternary ammonium compounds (QACs), 0.05% amine, and 0.025% peracetic acid (PAA)) on a stainless-steel surface in a laboratory setting using traditional cotton swab and the alternative sampling method. The alternative sampling method was further used to collect environmental samples (n=125) from two food processing lines for the detection of microbial load using Neogen® Rapid Aerobic Count Plates (RAC), Rapid *E. coli/* Coliform Count Plates (REC).

Results: The alternative sampling method showed higher recovery of both *E. coli* and *L. innocua* on stainless steel surfaces than a cotton stick. Recovery values of *E. coli* and *L. innocua* were 67.0±7.1% and 55.9±1.1%, respectively in the presence of 0.05% QAC; 83.9±1.3% and 59.5±8.7% in the presence of 0.05% amine, and 86.2±7.5%, and 43.9±4.2% in the presence of 0.025% PAA. The alternative sampling method also showed maximum recovery for total viable count (TVC) on food contact surfaces of 7.9 log CFU/100 cm^2 for stainless steel, 7.01 log CFU/100 cm^2 for nylon, and 7.2 log CFU/cm^2 for foam surface. Overall, the alternative sampling method showed higher performance in the recovery of TVC (3.4 to 7.7 log CFU/100 cm^2) and coliforms (2.6 to 7.4 log CFU/100 cm^2) compared to cotton stick after cleaning.

Significance: The alternative sampling method can be an effective tool for monitoring the bacterial load on food contact surfaces production facilities to verify and improve sanitation standard operating procedures.

P1-97 Analytical Method Development and Monitoring for Residual Solvents in Processed Foods

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Introduction: There are cases in which residual solvent test methods are not available even for foods for standards have been established. Therefore, it is essential to develop a reliable and accurate analytical method that can be applied to monitoring of residual solvents in processed foods for food safety.

Purpose: This study was aimed for development and validation a residue solvents method for 4 processed food(sugar, coffee, oil and tea) using Headspace gas chromatography/mass spectrometry (HS-GC/MS).

Methods: Benzyl alcohol was selected as the sample diluent and respective concentration were into a 20 mL headspace vial with 500 mg of sample (sugar, roasted coffee, olive oil and leaved tea). To verify the applicability of the develop method, selectivity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) was evaluated.

Results: The coefficient of determination (R^2) was above 0.99 within the concentration range from 0.2 to 10 μg/L. Recoveries were obtained by spiking standard solution (0.5, 2, 5 mg/kg) ranged from 86.6 to 113.8% for Methyl alcohol, 86.4 to 106.6% for Acetone, 91.3 to 108.5% for Isopropyl alcohol, 80.2 to

104.7% for Hexane and 88.8 to 105.2% for Ethyl acetate. And standard deviation values were less than 9.63% which is satisfied the AOAC guideline.

Significance: The results of this study demonstrated that the method is suitable for residual solvents in processed foods.

P1-98 Can UHT Skim Milk be Used for UV-C Validation Pilot Studies to Satisfy 403(h)(3)(B) of the Federal Food, Drug, and Cosmetic Act?

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Introduction: Thermal processing of skim milk for milk protein concentrate (MPC-85) manufacturing results in bioactive protein and enzyme degradation and damages product quality. Non-thermal technologies (UV-C) hold considerable promise for retention of nutritional and sensory quality. As per regulatory guidelines, raw skim milk should be used for microbial challenge studies, but this presents challenges due to background microflora interference with the validation testing.

Purpose: To evaluate whether ultra-high temperature processed skim milk (UHT-SM) could serve as a substitute for raw skim milk (RSM) in microbial validation studies during pilot trials.

Method: Two milliliters of UHT-SM or RSM, with 6 mm fluid thickness, was irradiated using collimated-beam device equipped with a low-pressure mercury lamp (12 Watts) emitting UV-C at 254 nm wavelength under stirred conditions. A series of known UV-C doses (0-6.9 $mJ \cdot cm^{-2}$) were delivered, considering all optical factors, to test samples inoculated with *Listeria monocytogenes* (ATCC 19115) at 7 log CFU/ml. For accurate measurement of light intensity radiometer was used. The experiments were conducted in triplicates followed by double plating and enumeration using selective media.

Results: The absorption and reduced scattering coefficients were calculated as 17.39±0.8 cm^{-1} and 17.02±1.08 cm^{-1}; 14.71±1.04 and 21.07±1.03 for UHTSM and RSM respectively. According to microbial kinetics, the exposure time needed for 1 log reduction of *Listeria monocytogenes* was 200 seconds for RSM. However, UHT-SM exhibited higher absorption, an exposure time of 243 secs was needed for similar inactivation. From this study, it is evident that inactivation studies of micro-organisms in UHT-SM require higher UV exposure-times as compared to RSM to inactivate the same number of micro-organisms. However, the dose required for 1 log inactivation was similar for both fluids.

Significance: The findings suggest UHT-SM is a suitable alternative for UV pilot-scale microbial validation studies for MPC production to satisfy (403(h)(3)(B) of the FD&C Act).

P1-99 Human-Centered Design of a Sensor-Enabled Decision Support System (SENS-D) for Creating a Safe, Equitable Food System

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Introduction: *Salmonella* is a One Health issue, and its incidence has not declined in three decades in the United States costing the economy $4.1 billion annually. Efforts to reduce salmonellosis and improve food equity will require a convergent approach through interdisciplinary teams and multisectoral collaboration to enhance data environments and build transformative technologies.

Purpose: The purpose of this study was to engage in interdisciplinary team science to determine the decision support needs of the poultry supply chain and health outreach stakeholders to mitigate *Salmonella* and create a safe, equitable food supply.

Methods: A three-phase human-centered design of low-fidelity prototypes was used, including in-depth interviews (n=16), gathering prototype feedback during mixed group 'think-tank' sessions during a workshop (n=32), and post-workshop interviews (n=7) (MU IRB#:2100184). Thematic and content analysis were conducted on qualitative data. Decision support needs and data sources important for food safety were prioritized to inform the development of a sensor-enabled decision support system (SENS-D).

Results: Poultry processors, a meat industry leader, and food banks reported the need for an affordable, rapid test to increase the timeliness of food safety decisions. Sensor placement and testing frequency was a problem stated by supply chain and health stakeholders. Consumer stakeholders, including a public health agency, Extension, and food banks reported there is a need for decision support to strengthen the workforce to target outreach to the highest risk populations facing food inequities. Another theme identified was a need for more analytical tools for evaluating the comparative efficacy of interventions and policies.

Significance: Creating integrated technologies that offer coordinated decision support across the food supply chain and health stakeholders has the potential to reduce food safety disparities. This preliminary work demonstrates there is value in combining multiple aspects of food safety efforts across supply chain and health stakeholders to detect and mitigate food-borne illness.

P1-100 Experts Roadmap Food Safety Short- and Long-Term Research, Technology, and Policy Needs for Cultured Meats

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Introduction: Research and technology advancements indicate that cultured meats (e.g., cellular agriculture, lab-grown meats) are a viable emerging food sector. Many unknowns remain about the biological and chemical hazards of these new products. Road mapping is a strategic planning technique widely used by industry, governments, and other organizations to develop and communicate strategy and innovation.

Purpose: The purpose of the food safety road mapping workshop was to bring together food safety experts to identify short- and long-term research, technology, and policy opportunities for cultured meats using a formalized road mapping strategy.

Methods: Twenty-three food safety experts from government, academia, and the private sector participated in a formal road mapping workshop at IAFP 2023. Participants divided into six groups to address (i) biological hazards and disease (two groups of four), (ii) chemical hazards, (iii) media, scaffolds, and inputs, (iv) risk communication, and (v) sanitation/SSOPs/equipment design. Groups used a roadmap template to define scope, identify desired outcome or change, define challenges and barriers, and identify short- and long-term solutions/opportunities and enabling environments to achieve the desired outcome.

Results: Each group identified distinct challenges and opportunities for their area of expertise (e.g., proprietary information makes regulation difficult, chemical media inputs not publicly characterized, opacity in input regulation, etc.). However, themes identified across groups included intellectual property protections slowing safety research and development, a need for a clear regulatory framework and safety standards, and consumer risk and impact on acceptance remaining undefined.

Significance: Food safety roadmaps identified discrete and systematic barriers and opportunities for cellular agriculture and offer a blueprint for short- and long-term research and technology investments and policy needs to ensure cultured meats are safe for consumption.

P1-101 Interventions to Control Human Pathogens in Hydroponic Crop Production: Scoping Review

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❖ ***Developing Scientist Entrant***

Introduction: Given the importance of food safety in controlled-environment agriculture, a comprehensive understanding of existing literature on effectiveness of food safety interventions is warranted.

Purpose: To map, characterize, and assess the quality of existing evidence on food safety interventions in hydroponic crop production and identify gaps in knowledge.

Method: A strategy was developed to search electronic databases (CAB Abstracts & Global Health, Web of Science, PubMed). Using pre-established criteria, (i) food safety, (ii) produce, (iii) soilless production, (iv) primary research studies were identified, and data (e.g., pathogen, produce, intervention type, methods, etc.) were extracted and collected.

Result: Of 93 relevant studies on hydroponic food safety, 24 reported on food safety interventions; n=20 papers were in English. *Salmonella* was investigated most frequently (n=10; 50%). *E. coli* O157:H7 was the focus of one-quarter of studies (n=5; 25%). Lettuce was the most studied produce (n=12; 60%). Chemical interventions were most investigated (n=12; 60%), focusing primarily on chlorine-based sanitizers (n = 7). The majority of studies (n=17; 85%) used a non-treated control to evaluate treatment effectiveness by comparison with the treatments, and 65% of the studies did not provide explicit details regarding the number of experimental replicates. Over one-third (n=7; 35%) of the articles did not state if sample replicates were used in their work. Despite stating that research is focused on hydroponic production, 60% of the studies (n=12) failed to disclose the type of hydroponic system used. Mitigation practices during transplanting, harvest, transportation, or retail were not investigated, highlighting the gaps in the published evidence.

Significance: The findings provide a map of intervention studies, creating a repository of information that will serve as a foundation for future systematic reviews, provide gaps to be addressed in future work, and inform policy and practice.

P1-102 National Surveillance of Microbial Indicators and Foodborne Pathogens in Commercial Beef, Pork and Poultry Processing Facilities in South America

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Introduction: Regulatory frameworks depend on national data collection systems for developing performance standards and assessing changes in contamination levels in different commodities. Data analysis of microbial surveillance programs is necessary to communicate risk to processors and consumers, and to identify data trends that can support the implementation of regulatory performance standards.

Purpose: To develop a microbial indicator and pathogen prevalence baseline benchmark for processors and stakeholders based on surveillance data collected in different commercial beef, pork, and poultry processing facilities in a South American country.

Methods: Samples were collected by trained inspection personnel throughout a 60-month period of operations to account for variability. The samples were weighed (25g) and 225mL of buffered peptone water (BPW) was added. Homogenates were stomached and serially diluted. The samples were analyzed for *Salmonella spp*. prevalence and quantification of generic *Escherichia coli* using standard methodologies.

Results: Overall, for *Salmonella spp*., beef samples had a very low prevalence with the highest prevalence of 4% on the third year. However, poultry meat showed the highest prevalence of 12.67% for the first year and 11.11% for the third year. Pork meat showed a prevalence of 3.91% in the fifth year. For *E. coli* quantification, beef showed an average of 5.26 log CFU/10mL with the first year having the highest counts. For poultry, an average of 4.87 log CFU/10mL was quantified with the first year having the highest counts, similar to beef. Pork meat showed the highest quantification with an average of 6.55 log CFU/10mL.

Significance: This study can be used as a monitoring system to identify the risks associated with pathogens and indicators in meat and poultry products. Data showed there was a high prevalence of Salmonella spp. throughout five years. The quantification of generic E. coli revealed minimal fluctuation throughout the course of the year for all three products. Intensified sampling is needed to support the development of regulatory performance standards.

P1-103 Evaluation of Process Wash Water Microbial Quality and Physicochemical Variables in a North Carolina Fresh-Cut Leafy Green Operation

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Poster

Introduction: Peracetic acid (PAA) is a commonly used sanitizer for controlling pathogen cross-contamination in fresh-cut leafy green process wash water. While routinely monitoring the concentration of PAA indicates potential cross-contamination control, measurements face challenges.

Purpose: The purpose of the study was to evaluate the efficacy of a recirculated wash water system using PAA in a fresh-cut leafy greens facility based on relationships between physicochemical variables and microbial load.

Methods: Samples of water (100mL) from two tanks continuously dosed with PAA (n=63), pre-wash and post-wash (n=54) leafy greens (25g) were collected. Plate count agar, EC Petrifilm, and Colilert Quanti-Tray/2000 were used to measure aerobic plate count (APC), total coliforms, and *E. coli* per 100mL of the water samples. Pre-wash and post-wash produce were assessed using plate count agar and EC Petrifilm. Data was analyzed using Pearson correlation coefficients and simple linear regressions ($p<0.05$).

Results: Total average and standard deviations of PAA concentration was 5.2 ± 5.5 ppm in tank 1 and 17.2 ± 10.6 ppm in tank 2. The average microbial load was greater in tank 1. Temperature was positively correlated with microbial load (r=0.80 with APC, r=0.96 with total coliforms) and negatively correlated with PAA concentration (r=-0.76) in tank 1. PAA concentration and oxidation-reduction potential (ORP) were significant explanatory variables for total coliforms and APC in both tanks. *E. coli* was greater than 0.3 log CFU/100mL in tank 1 when the PAA concentration was below 2 ppm. There was no significant difference between pre-wash and post-wash leafy green microbial load.

Significance: Results suggest dosing amounts may be optimized to maintain adequate PAA concentrations based on expected water microbial load and temperature. Findings underscore the significance of monitoring produce wash water as a potential pathogen environment and cross-contamination point in fresh-cut processing.

P1-104 Prevalence of Constitutive Microflora Isolated from a Lettuce Hydroponic Nutrient Film Technique System

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❖ ***Developing Scientist Entrant***

Introduction: Controlled environment agriculture (CEA) production systems, such as a hydroponic system have been experiencing rapid growth. The application of this system also serves as a nutrient source for a variety of microorganisms.

Purpose: This study examined the prevalence of constitutive microflora of the biofilm isolated from a Hydroponic Nutrient Film Technique (NFT) system.

Methods: Sponge swabs were used to collect samples from the nutrient contact surface from a hydroponic system from a lettuce farm. Samples were swabbed in duplicates from a) hydroponic tubing, (b) water pump, (c) drainage area, and (d) reservoir. All the samples were plated on tryptic soy agar and incubated for 24 h at 37°C. The isolates were later identified using Matrix Assisted Laser Desorption Ionization-Time of Flight Spectroscopy (MALDI-TOF). Identified species were further used for co-exclusion study. A total of 120 combinations of two isolates spiked at a 1:1 ratio was prepared in tryptic soy broth, incubated at 37°C for 24 h, followed by plating on tryptic soy agar. The isolates on plates were distinguished based on colony morphology, gram staining, and MALDI-TOF. For establishing the predominance, plate counts were compared using analysis of variance.

Results: The culturing techniques used to isolate constitutive microflora revealed the presence of gram-positive as well as gram-negative bacteria. MALDI-TOF identification showcased the presence of *Enterobacter bugandensis, Lysinibacillus sphaericus, Bacillus megaterium*, *Pseudomonas koreensism, Pseudomonas otitidis, B. cereus, M. luteus, B. infantis, Aeromonas hydrophila, Chryseobacterium gambrini, Lysinibacillus fusiformis, Exiguobacterium indicum, Pseudomonas putida, Kocuria marina,* and *Rossellomrea* spp. All the cultures were combined with different combinations of 7-8 log CFU/mL. In all the co-culture combinations, *Bacillus megaterium* and *Bacillus cereus* emerged as predominant species from the constitutive microflora.

Significance: This study provides insight into the constitutive microflora that would be further applicable in developing effective antimicrobial strategies.

P1-105 Interactive Food Safety Training Tool: Research Study With Small Food Operations

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Introduction: Small processing facilities and commercial incubator kitchens have created opportunities for processors to produce value-added products. The end users of these spaces are from diverse backgrounds with a passion for their products. Despite that, they often lack food safety training tailored to the needs of their small operations. This training limitation often leads to regulatory compliance issues related to food safety practices. A multistate food safety extension specialist team developed an interactive educational technology module.

Purpose: In this presentation, we will share data from a research pilot study. The research study collects summative data to evaluate the impact of the interactive module on small food processors.

Methods: We demonstrated the interactive module at local food conferences and meetings, public health conferences, and shared-use kitchens. Following the demonstration, participants completed a Qualtrics survey designed as a retrospective pre- and post-survey. The survey measures participants' experience with the interactive module and measures participants' perceptions of knowledge gained about food safety practices.

Results: As preliminary data (n=9), the pilot identified the module as helpful for participants' work responsibilities (maintenance, food safety, food production). Overall, after using the interactive module, participants had an increased perception of knowledge in the following aspects of food safety: "How to use personal protective equipment to keep food safe", "Proper hygiene protocols for food safety", "Standard Operating Procedures for food safety"; "Record keeping procedure to maintain food safe environment". Additionally, our team learned the need to add audio narration to the module to support users with low reading skills and to have the module available in Spanish.

Significance: Building an engaging and research-based educational technology helps make food safety training accessible and effective. The module enables more robust access to training tools for under-served communities with limited resources to help improve food safety practices. The full summative retrospective survey data with a larger and significant sample will be presented during this session.

P1-106 Environmental Survey of *Enterobacteriaceae* and Total Aerobic Counts on Elementary Students' Lunchboxes Utilizing Rapid Quantification Technologies

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Introduction: Newly developed rapid quantification methodologies have many applications for food safety. While these methods are commonly implemented within a processing plant, it is important to study and further understand their possible applications for food safety and public health at the consumer level.

Purpose: The purpose of this study was to utilize Hygeina® Microsnaps® to survey APC and EB populations found on elementary students' lunchboxes and determine if properties such as staining, or smell correlated with the observed bacterial loads.

Methods: 15 lunchboxes were obtained from students at a public elementary school in Lubbock, Texas. Each of the lunchboxes was assigned a random 3-digit ID, and the physical properties of the container, such as material, smell, and staining, were recorded. Then, each lunchbox was swabbed using the Hygeina® Microsnaps® for APC and EB, these swabs were then transported to the ICFIE microbiology laboratories and processed according to their respective protocols.

Results: The results of this study indicate that the lunchboxes that were noted as having a detectable odor or were constructed of a traditionally "difficult to clean" material, resulted in higher counts of both APC and EB. 7 of the 15 observed samples were recorded as "clean in appearance" with "no detectable odor", each of these samples reported <10 log CFU/Sample of both EB and APC. However, samples that were reported as "stained" or having a "strong odor" produced counts of EB and APC that ranged from 3 to 5 LOG CFU/Sample. Additionally, this study indicated that the two counts correlated with one another, suggesting that APC may be utilized as an indicator of potential EB counts.

Significance: The data obtained in this study can be utilized by schools to recommend cleaning lunchboxes to prevent foodborne illnesses. Additionally, it illustrates that rapid detection and quantification methodologies can be applied in many environments for future studies.

P1-107 An Approach to Estimate Underdiagnosis Multipliers for Foodborne Illnesses in the United States

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Introduction: Estimates of the number of foodborne illnesses are valuable for prioritizing food safety interventions. However, underdiagnosis occurs if any of four factors exists: the ill person does not seek medical care, a specimen is not submitted for laboratory testing, the laboratory does not test for the causative agent, or the test does not identify the pathogen.

Purpose: Develop statistical models to estimate multipliers accounting for underdiagnosis for select pathogens in the Foodborne Diseases Active Surveillance Network (FoodNet).

Methods: Bayesian models were developed to estimate probabilities of medical care seeking and specimen submission for individuals who had acute diarrheal illness (n=1,547) using demographic and symptom information from the 2018–2019 FoodNet Population Survey and applied to cases from 2017–2019 FoodNet surveillance (n=29,147). The FoodNet Laboratory Survey was used to estimate the probability of laboratory testing by site and year. Bootstrap samples were drawn from the estimated probabilities. Sensitivity of culture against culture-independent diagnosis test (CIDT) was estimated using CIDT positive surveillance cases with reflex culture (n=11,358) and used as the low value for a PERT distribution from which random draws were selected. The reciprocals of draws (n=10,000/factor) for the four factors were multiplied to obtain an underdiagnosis multiplier distribution.

Results: For *Campylobacter*, *Salmonella*, and Shiga toxin-producing *Escherichia coli* (STEC) O157 and non-O157 STEC, the probability of seeking medical care ranged from 0.39 to 0.50 and the probability of submitting a specimen ranged from 0.10 to 0.15. Almost all submitted specimens were tested (range: 0.90 –1). Sensitivity of culture ranged from 0.66 to 1. Overall, underdiagnosis multipliers ranged from 39.0 to 52.7.

Significance: Our innovative approach that predicts probabilities at the individual level and accounts for test sensitivity using CIDT and reflex culture data could improve estimates of foodborne illnesses in the United States.

P1-108 Effectiveness of a Behavior-Based COVID-19 Risk Management Training Program: A Mixed Methods Study on Prevention Practices for the Safe Reopening of Food Service Businesses in North Carolina during the Post-Pandemic Era

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❖ ***Developing Scientist Entrant***

Introduction: Restaurants and lodging businesses provide 531,000 jobs with $27.3 billion in sales yearly in NC (NCRLA, 2023). Count On Me NC offered behavior-based COVID-19 management training to 5,580 managers and 9,312 food handlers.

Purpose: To quantitatively and qualitatively assess training effectiveness for safe reopening of food businesses.

Methods: This convergent-parallel mixed methods study investigated managers' (n=203) and food handlers' (n=99) self-reported behaviors for COVID-19 risk management using online surveys (n= 302) and in-person interviews (n=8). The 5-point Likert scale, cross-sectional survey, based on the Integrated Behavioral Model, was administered one year after the training. Behaviors included: physical distancing, use of masks, staff staggering, COVID-19 symptoms awareness. Quantitative data were analyzed using JMP Pro 15 Version 15.2. T tests (CI=90%) determined significant differences in respondents' scores. Interviews were done through convenience sampling. Thematic content analysis explored participants' COVID-19 risk management experiences at work. Data was integrated and triangulated to assess training effectiveness.

Results: The Count On Me NC training program was effectively delivered across factors: types of restaurants (local versus chain), geographic location (Metropolitan versus Micropolitan), and risk-based prevention measures (physical distancing, use of masks, staggering of shifts, symptom awareness). Quantitative data revealed overall Likert scores tended towards "Strongly agree" to implementing the majority of COVID-19 recommended prevention measures. Qualitative data (interviews) showed that use of masks and cleaning and sanitation were more commonly implemented (80% of responses) due to the perceived control managers and food handlers reported. There were no significant differences when scores were compared across factors. Sample size and location limit the generalizability of these findings.

Significance: Count On Me NC addressed all COVID-19 prevention measures. Self-reported implementation of prevention measures concluded that the program effectively supported food service businesses to safely reopen. Behavior-based training is a viable option to develop effective learning material during a global pandemic.

P1-109 Investigating the Microbial Quality of Food Products Obtained from Farmers' Markets in Central Virginia amidst the COVID-19 Pandemic

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Introduction: Considering the positive correlation between the number of farmers' markets and reported cases of foodborne illness, it is essential to examine whether the mandated use of PPE, such as masks and gloves by stakeholders during the COVID-19 pandemic, has influenced the microbial quality of food products obtained from farmers' markets in Central Virginia.

Purpose: This study evaluated the prevalence and antimicrobial resistance (AMR) of bacteria in potential at-risk products identified by the VSU preliminary study during the COVID-19 pandemic when stakeholders adhered to PPE protocols while handling contact and non-contact food products.

Methods: A total of 740 food items were collected for microbial quality analysis. These items were sourced from 76 farm operations and distributed across 15 officially registered farmers' markets in Central Virginia between August 2020 and December 2021.

Results: Despite the use of PPE, *Campylobacter*, *E. coli*, *Listeria*, and *Salmonella* were detected in the samples at rates of 1.5%, 19.2%, 7.3%, and 0.8%, respectively. Compared to the period between March and November 2017 (before the COVID-19 pandemic), the prevalence of *Campylobacter* and *Salmonella* increased by approximately 6.2% and 0.5%, respectively, while *E. coli* and *Listeria* decreased by 0.3% and 4.9%, respectively. Among the bacterial isolates, resistance to ampicillin, streptomycin, nalidixic acid, and amoxicillin-clavulanic acid was observed in *Campylobacter*, *E. coli, Listeria*, and *Salmonella* isolates, respectively. At least one isolate of each bacteria was non-susceptible to seven antibiotics. Streptomycin resistance was the most common, found in 197 (48.8%) isolates, and approximately 7% of the bacterial isolates exhibited multidrug resistance (MDR). None of the tested antimicrobials was effective against all bacteria.

Significance: The findings of this study highlight the occurrence of potential foodborne pathogens regardless of the implementation of PPE. Continued research is needed to determine and intervene in the cause(s) of the observed prevalence and to support the healthy development of food products sold at farmers' markets.

P1-110 Regulatory Considerations for Small-Scale Produce Drying Operations: A Multi-State Perspective Obtained Through Interviews with Inspectors

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❖ ***Developing Scientist Entrant***

Introduction: Increasing recalls implicating foodborne pathogens with dried produce has demonstrated a need to assess current food safety implications of produce drying. Specifically, small-scale produce drying processors which face barriers to successfully complying with food safety regulations.

Purpose: To identify food safety education needs for small-scale produce drying stakeholders and to better understand the inconsistencies among regulatory oversight for small-scale produce drying.

Methods: Participant recruitment was conducted on a multi-state level – through local points of contact. We interviewed 32 food safety inspectors from 15 states via Zoom, using a script that three university food safety specialists had reviewed and three regulatory professionals had pilot-tested.

Results: The study revealed discrepancies among inspectors in "what to inspect" and "what to follow." Inspectors gave varying responses regarding safe water activity levels. Some inspectors looked for document records, the specific documentation type requested varied, including HACCP (Hazard Analysis and Critical Control Points) plans, SOPs (Standard Operating Procedures), and worker certifications. The study revealed a lack of uniformity in which regulations or rules were followed for inspections. Although the FDA Food Code was most mentioned, participants' version varied significantly among each regulator. Additionally, inspectors reported complications in inspecting small and very small produce drying facilities. First, inspectors expressed frustration with regulations related to drying, as they felt the language was considered vague, too long, or outdated. Second, inspectors struggled to explain the importance of certain food safety rules to processors in a way they understood. Third, insufficient and readily available educational resources frustrated inspectors, several of whom complained about underfunding of public health departments, an inadequate workforce, and inadequate appropriate training for inspectors.

Significance: The findings identify a need for clarity on food safety standards in produce drying and an increased levels of technical and educational support for small-scale produce drying stakeholders.

Poster

P1-111 Prevalence of Common Foodborne Bacteria in Retail Poultry Meat In Hawaii

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Introduction: Systematic surveillance of foodborne pathogens in retail poultry meat products is essential for implementing intervention strategies to minimize contamination.

Purpose: The objective of this study was to determine the prevalence of four common foodborne bacteria (*Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus*) in chicken and turkey meat products sold in Hawaii, collected by the National Antimicrobial Resistance Monitoring System (NARMS) program.

Methods: Ninety chicken and 90 ground turkey samples were purchased from randomly selected grocery stores in Hawaii in 2020. Associated information (e.g., production claims, package types) of each sample was also recorded. Samples were tested for *Salmonella, Campylobacter*, *E. coli* and *Enterococcus* following the NARMS Retail Meat Isolation Protocol. Fisher's exact test and post hoc analysis were performed using R-studio to determine any significant differences in prevalence among different types of meats.

Results: The prevalence of *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus* was 14.4%, 7.22%, 67.7%, and 72.2% respectively. Overall, *Salmonella* and *Campylobacter* prevalence was higher (p<0.05) in chicken, while *E. coli* and *Enterococcus* were more (p<0.05) prevalent in turkey samples. Additionally, bacterial prevalence did not (p>0.05) differ among cuts of meat, except for a higher prevalence of *Enterococcus* observed in chicken breast, mixed parts, and whole chicken samples. A significantly higher (p=0.02) prevalence of *Salmonella* was found in "antibiotic-free" labeled meat products. Meat packaged in stores showed a higher (p=0.04) prevalence of *E. coli* compared to packaged-ready meats. No difference (p>0.05) was observed in the prevalence of all four bacteria among different seasons, organic claim status, and package types.

Significance: The study results underscore the importance of following proper handling and cooking practices to minimize the risk of foodborne bacterial transmission and highlight the significance of ongoing pathogen monitoring conducted by the NARMS.

P1-112 Bacteriological Safety of Ready-To-Eat Chicken and Food Contact Surfaces in Restaurants at a South African University Campus

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Introduction: Globally, microbial contamination of food plays a significant role in foodborne illnesses, with one in ten people being affected every year.

Purpose: This study aimed to determine food hygiene and safety risks by examining the bacteriological safety of ready-to-eat (RTE) chicken and food contact surfaces in restaurants located at a South African university campus.

Methods: A cross sectional quantitative study design was used, and 426 samples were collected from six restaurants. Samples were analyzed for Total Viable Counts (TVCs), *Escherichia coli*, Salmonella, and *Staphylococcus aureus* using standard ISO test methods. Only food handlers who were involved in the processing of RTE chicken were selected (5 food handlers per restaurant). The samples were collected from the dominant hands (palm, thumb, forefinger, and middle finger; n=150); aprons (10 cm^2; n=210); food contact surfaces (10 cm^2; n=42); and RTE chicken (100 g; n=24).

Results: TVCs that exceeded the national specified limit of 1 x 10^2 $cfu.cm^{-2}$ were detected as follows: 5 (17%) on food handlers' hands; 6 (20%) on food handlers' aprons; and 1 (17%) on direct food contact surfaces. The RTE chicken TVC results were within the national specifications of less than 10 000 TVCs per gram. Facility three recorded the best TVC national specification compliance rate with no TVC failures, while facilities two and five recorded the highest TVC non-compliance rate with four TVC failures each. No *Escherichia coli*, Salmonella or *Staphylococcus aureus* were detected on the analyzed food contact surfaces and RTE chicken samples. A statistically significant difference (p<0.05) between the number of TVCs was detected when apron- and hand samples were compared (p=0.012 for aprons).

Significance: This study demonstrated that hygiene levels of food contact surfaces were not in compliance with national specifications. Consequently, improvements need to be implemented to increase personal and general hygiene levels to ensure food safety.

P1-113 Food Allergy Labelling and Accommodation on Non-chain Restaurant Websites and Menus in Toronto, 2023–2024

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Introduction: Restaurants have a responsibility to mitigate food-allergic reactions by non-verbally disclosing food allergens on their menus and websites.

Purpose: To assess the prevalence of allergen labeling on online websites and menus of non-chain restaurants, emphasizing its importance as a crucial factor in customer awareness and accommodation.

Methods: This quantitative cross-sectional study utilized a random sample of 1,000 restaurants sourced from DineSafe, Toronto's inspection system. Selected restaurants' online websites and menus were assessed using a checklist designed to capture general information (cuisine type, Google rating, cost indicators) and to determine specific details pertaining to food allergen labelling (separate allergen menu, allergen statement, menu symbols for major allergens). Chain restaurants were removed from the dataset prior to coding. Geolocation data of selected restaurants will be mapped to spatially visualize allergen labelling results based on City of Toronto's 2016 neighbourhood census demographic data.

Results: Only 10% of the 1,000 analyzed restaurants featured one or more allergen symbols (n=100). The most listed allergens were gluten (7.5%), followed by nut-related symbols (3.8%). Moreover, 1.7% of restaurants had dairy symbols, and 1% had seafood symbols. Additionally, 0.4% of restaurants denoted sesame, 0.3% denoted soy, and 0.2% denoted eggs. Another 8% of restaurants lacked symbols but offered gluten-free options and substitutions upon request. Only six restaurants had a separate gluten-free menu/sub-section, and four restaurants had a separate allergen list. Approximately 16% of restaurants provided allergen disclosures, with 1.2% labelling seven or more allergens, 5% some, and the remainder offering a general statement that customers should inform servers of any allergies. Further insights into the relationship with neighborhood socio-economic indicators will be gained through GIS analysis.

Significance: These results indicate a lack of food allergy accommodation in non-chain restaurants in Toronto. Data can inform government and food allergy outreach organizations, enabling them to implement interventions such as increased food allergen training for employees and enhanced allergen labeling on restaurant menus.

P1-114 Assessing Consumer Perceptions of Food Safety in Mobile Eateries: A Study on Food Trucks

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✦ ***Undergraduate Student Award Entrant***

Introduction: The growing popularity of food trucks has coincided with an uptick in reported cases of foodborne illnesses. Food trucks frequently operate under minimal oversight and in varying conditions, factors that have likely contributed to an increased incidence of foodborne illness outbreaks. However, there are scant studies examining consumer perceptions of food safety in food trucks.

Purpose: The aim of this study is to explore how consumers' food safety knowledge, perceived service quality, past experiences with food trucks, and perceived risk are related to their perceptions of the safety of food served from food trucks.

Method: A total of 286 respondents, who had ordered food from food trucks in the United States within the past six months participated in the survey via Prolific. Descriptive statistics and multiple linear regression were used for data analysis.

Results: Participants in our study comprised 45.1% males and 54.5% females. Approximately 80% of respondents were aged between 18 and 39. In the multiple linear regression analysis, the determination coefficient (R^2) was 0.398. The results revealed significant positive correlations between food truck customers' knowledge about food safety ($\beta = 0.088$, $p < 0.05$), perceived service quality ($\beta = 0.215$, $p < 0.01$), and prior experience ($\beta = 0.291$, $p < 0.001$) with their perceived safety of the food. Conversely, customers' perceived risk ($\beta = -0.289$, $p < 0.001$) was negatively associated with perceived food safety from food trucks.

Significance: Our findings provide valuable insights for food truck operators. By comprehending the factors that shape customers' perceptions of safety, food truck vendors can implement targeted strategies that enhance consumer confidence during their visits to food trucks.

P1-115 Enhancing Food Safety Concepts among Food Handlers through Educational Interventions

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Introduction: Developing Food Safety (FS) concepts and incorporating the concepts of risk and hazard by food handlers is crucial to evolve risk perception.

Purpose: To enhance FS concepts among food handlers.

Methods: The research took place in a food service in São José dos Campos, Brazil, involving 36 food handlers. A FS-culture diagnosis was performed using a mixed method assessment (quantitative and qualitative), and each FS-culture element scored from one to three. The element with the lowest score was used as a starting point for educational intervention. In the intervention, participants were asked to individually define FS-related concepts (hazard, FS, risk, or foodborne disease) on paper. Subsequently, participants were divided into four groups, each assigned one concept for discussion and presentation. A guided discussion ensured a structured research process. To evaluate the intervention, the concept progress was analyzed qualitatively. The Brazilian Ethics Committee approved the project (0276/2022).

Results: The mean age of participants was 31 years (Standard deviation: 11.91), and 100% were men. FS-culture scored 2 (active), and Risk perception was the element with the lowest score (1-2, reactive to active). Before group discussions, the definitions provided by food handlers in the paper were inadequate compared to the literature, except for foodborne disease. Hazard was described as food poisoning due to accidents, FS encompassed aspects of food handling, while risk was linked to diseases transmitted via contaminated water and food. Post-discussion, these concepts evolved towards more accurate representations. Hazard was redefined by the food handlers as a chemical, biological, or physical agent capable of causing injury or death, risk was defined as the likelihood and consequences of an event, while FS was understood as ensuring food does not adversely impact consumer health.

Significance: The educational intervention improved FS concepts among food handlers, which may evolve FS-culture. Knowing these concepts is essential for developing risk perception in food handlers, improving FS and positively impacting public health.

P1-116 Willingness to Pay for New Sanitation Technologies in Food Industries: a Comprehensive Choice Analysis of Purchasing Preferences among Manufacturers

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❖ ***Developing Scientist Entrant***

Introduction: Surface sanitation in food industries involves technology used, chemical needs, labor, and costs. Innovation is always evolving to make sanitation more efficient, less costly, and environmentally friendly.

Purpose: This IRB approved research aims to identify the key determinants of industry preferences for novel sanitation technologies according to their perceived attributes.

Methods: A two-part online questionnaire was distributed to food industry representatives. Section one included sociodemographic and recall questions. Section two included a discrete choice experiment examining the impact of attributes of novel sanitation technologies on participants' willingness to pay. A set of ten choice tasks was presented to each respondent based on their main concern: Pathogen or Spoilage. Attributes were purchasing cost, microbial reduction, environmental *Listeria* positives (ELP), spoilage reduction rate (SR), water and chemical use, maintenance, and effect of wear and tear. A mixed model regression was utilized to analyze the choice data.

Results: A total of 263 responses were collected with 7401 observations. The variable price was negative and statistically significant ($p<0.01$), suggesting that as the price increases, the purchase likelihood for the new technology decreases. Among attributes, environmental *Listeria* positives and spoilage rate were the most significant ($p<0.01$ in all ELP variables, and between $p<0.01$ to $p<0.1$ for SR) when making a purchasing decision. A significant preference in microbial reduction of 99.9999% ($p<0.01$) and a maintenance price of \$2,000/year ($p<0.01$) relative to 90% and \$20,000/year, respectively, was found. Results showed that 28.5% of food industry respondents had experienced a recall, and they were willing to pay more for a 99.99% microbial reduction, 0.1% and 1% of ELPs, labor and maintenance costs, compared to industries that have not experienced a recall.

Significance: The present study can provide valuable insights for stakeholders involved in sanitation infrastructure development and can help in designing cost-effective and socially acceptable solutions for improved sanitation practices.

P1-117 Evaluation of Occupational Safety Risks and Perceptions by Human Subjects in the Manual Application of Superheated Steam as a New Surface Sanitation Technology in Food Processing Industries

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Introduction: Superheated steam is a surface sanitizer that shows potential in food industries that do not use water in sanitation. The temperature of superheated steam is ≥125□C and it is invisible to the naked eye, which might pose a risk to operators.

Purpose: This study assessed operators' perception of occupational safety risks associated with manual superheated steam delivery through a commercial scale equipment.

Methods: An IRB approved trial with 24 human subjects was conducted. A safety training was administered prior to the subjects' hands-on use of the superheated steam tool. A survey was presented to the subjects before training, after training, and after the use of the tool. Descriptive statistics were calculated, and paired and independent t-test were performed.

Results: Respondents' median age was 26 years old, 70% were female and 30% male. Results showed that 46% considered the time and accuracy needed to operate the tool as the biggest limitation with a mean of 1.6 ± 0.64 (1: most important, 4: least important). The weight of the equipment was considered the second greatest limitation (mean: 2.1 ±0.92), and employee safety was the third (mean 2.5 ± 0.97). The amount of personal protective equipment was considered the least important limitation (mean of 3.8 ±0.38). Before the trial, seven respondents found the technology "very dangerous" or "dangerous," and no respondents perceived it as "safe." After hands-on implementation and training, six respondents still viewed it as "very dangerous" or "dangerous", although an increase in the "not very dangerous" (n=8) and "safe" (n=2) was noted. T-tests showed no significance.

Significance: These findings shed light on the potential challenges associated with manual operation of superheated steam technology, offering insights for further improvements of the design of the tool. This work is an example of the type of translational research needed to support broader adoption of novel technologies.

P1-118 *P. aeruginosa* Cross-Contamination is Significantly Impacted by Material Type and Active Ingredients in Cleaning and Sanitation

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❖ ***Developing Scientist Entrant***

Introduction: Optimization of wiping materials and products used in cleaning and sanitizing is crucial to mitigating the spread of pathogens on food contact surfaces. Limited data is available on cross-contamination risks of wipe substrate and chemistry combinations.

Purpose: The purpose of this study was to evaluate cross-contamination risk from different wiping materials paired with various chemistries to surfaces previously not contaminated with *P. aeruginosa* (e.g., gloves).

Methods: Three wipe materials impregnated with water, quaternary ammonium, hydrogen peroxide, and ethylated alcohol cleaners were tested against *P. aeruginosa* (ATCC 15442) on Formica to determine the bactericidal efficacy of disinfectants under conditions mimicking real-world use. The Formica boards were inoculated with approximately 7 log CFU. Boards were dried for one hour after inoculation. The board was wiped for one meter before swabbing a 10x10 cm sampling zone (World Bioproducts LLC) after defined label contact time. Following the wiping procedure, the glove was imprinted on tryptic soy agar (TSA; Fisher Scientific) for five s then discarded. The plates were incubated for 24 h at 37°C to evaluate the cross-contamination risk using different wiping materials and chemistries.

Results: Each material and chemistry combination showed evidence of cross-contamination to the gloves. Nonwoven towelettes transferred significantly less *P. aeruginosa* across the surface than other material types tested in the study (p=0.017). Within the inoculation zone, the hydrogen-peroxide and quaternary ammonium-based products showed significantly higher levels of bactericidal activity compared to the other chemistries evaluated (p=0.0014). When assessing the cross-contamination risk across the board, there was no significant difference in bacterial load transferred to the sampling zone among the chemistries tested (p=0.2102).

Significance: Products and wiping material combination should be considered in sanitation SSOPs. Opportunities remain to improve SSOPs for gloves as they may be a source of cross-contamination if not properly disposed of after use.

P1-119 Evaluating Inactivation Strategies for *Listeria monocytogenes* on Enoki Mushroom (*Flammulina velutipes*) Contact Surface

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Introduction: Hygienic practices are crucial to prevent cross-contamination and biological hazards in enoki mushroom industry.

Purpose: This study aimed to determine the effects of sodium hypochlorite and peracetic acid, alone and in combination with thermal treatment, on *L. monocytogenes* on the contact surfaces used during enoki mushroom processing.

Methods: The surfaces such as conveyor belts, stainless steel, velcro strips of bottle wrapper inoculated with *L. monocytogenes* were immersed in chlorine dioxide (200 mg/L), sodium hypochlorite (200 mg/L), peracetic acid (200 mg/L), and chlorine dioxide (200 mg/L) solution at room temperature for 5, 10, 30, and 60 min. Then, the coupons were subjected to dry sanitization at 50, 60, 70, and 80 °C for 2 h for synergistic effect of chemical disinfectant and thermal treatment.

Results: This study evaluated the efficacy of chemical disinfectants and thermal treatments, alone and in combination, on various contact surfaces including conveyor belts, stainless steel, velcro strips of bottle wrapper. The results indicate that sodium hypochlorite, peracetic acid, slightly acidic electrolyzed water, and chlorine dioxide effectively reduced *L. monocytogenes* on clean surfaces, with stainless steel showing the greatest reduction. Whereas, the presence of mushroom debris affected the disinfectant efficacy, but sodium hypochlorite showed performing well on stainless steel even in the presence of organic matter. Peracetic acid emerged as highly effective, especially on conveyor belts. Combining chemical disinfectants with dry heat treatment, particularly peracetic acid, showed synergistic effects on velcro strips. Especially, steam treatment at 70°C was insufficient alone, but when combined with subsequent drying, it effectively reduced *L. monocytogenes* levels.

Significance: These findings suggest that tailored strategies combining chemical disinfection, and drying steps are effective in eliminating *L. monocytogenes* contamination on various surfaces encountered in mushroom processing.

P1-120 Assessment of Three Key Aspects to Enhance Food Safety Programs in Papaya Production

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Introduction: American associations and the Mexican government have issued guidelines to prevent contamination during papaya production. However, producers have expressed uncertainty regarding the implementation. Examples include determining the appropriate location on the papaya for monitoring temperature, the potential transmission of heavy metals through harvesting paper, and the impact of residual post-wash humidity on microbial load.

Purpose: To evaluate three methods of temperature monitoring in papayas, the effect of humidity on microbial load, and the potential transmission of heavy metals through harvesting paper.

Methods: In packaging facilities, the temperature of papayas (n=756) was measured on the epicarp with a laser thermometer and on the pulp with a stem thermometer. Lead, mercury, and arsenic were analyzed by atomic absorption in harvesting paper from 18 companies across seven states in Mexico. Papayas were also wrapped under three humidity conditions with harvesting paper containing detected lead residues and left to rest for 8 hours. The epicarp and pulp were analyzed using atomic absorption. In the laboratory, the papaya washing procedure was replicated (n=12). The fruits were packed with residual humidity or left to dry at room temperature, stored for 72 hours at 20°C, and aerobic plate count, *Enterobacteriaceae*, and molds-yeasts were determined using Petrifilm®. Results were compared through analysis of variance (α=0.05) using Statgraphics19.

Results: The temperature measured on the epicarp was 0.5°C lower than internal measurements (p=0.0469). Lead levels of 2.060, 0.828, and 2.420 mg/Kg were detected in three samples of harvesting paper; however, the epicarp and pulp tested negative. No significant difference in the microbial load (≤1 log CFU/100cm^2) was observed concerning post-wash residual humidity (p=0.6293, 0.8327 and 0.8890 for each indicator, respectively).

Significance: These results will support producers in substantiating their procedures based on scientific evidence, as well as assist organizations in drafting guidelines that emphasize actions posing higher risks to safety.

P1-121 Methods for Evaluating Cleaning and Disinfection Procedures of Contact Surfaces in an Avocado Packing Plant

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Introduction: The effectiveness of cleaning and sanitizing (C&S) procedures can be determined through counts of indicator organisms, visual inspection, or measurement of residual ATP. The industry must assess the advantages and disadvantages of each method to incorporate them into the monitoring and verification of their standard sanitation operating procedures.

Purpose: To compare three methods for monitoring and verifying C&S of food contact surfaces (FCS) in an avocado packing plant.

Methods: A total of 161 samples of FCS were tested before and after C&S procedures. Each surface was evaluated by visual inspection, scoring the dirt level from 1-4 (1 being acceptably clean and 4 being maximum dirt). Simultaneously, swab samples were collected for microbial and residual ATP testing.

In the laboratory, aerobic plate count (APC), *Enterobacteriaceae,* molds, and yeasts were enumerated using Petrifilm™. Data were analyzed by analysis of variance and correlation (α=0.05), and by Chi^2 for visual scale scores.

Results: Counts of APC, *Enterobacteriaceae,* molds and yeasts were 2.9, 2.0 and 1.6 Log_{10} CFU/100 cm^2 before C&S, respectively, whereas after C&S, these numbers were 4.0, 2.2, 1.4, and 1.4 Log_{10} CFU/100 cm^2, respectively. Counts were significantly different (p<0.05) for APC, *Enterobacteriaceae* and molds. ATP measurements before and after C&S were 4.8 and 3.0 Log_{10} RLU/100cm^2, and these values were not significantly different (p>0.05). For visual inspection, the percent samples with dirt scores 3-4 before and after C&S was 73% and 24%, respectively (p<0.05). A significant difference and a weak correlation were observed between visual scale levels and microbiological indicators, but not between visual scores and ATP.

Significance: C&S procedures produced minimal or null reduction in microbiological indicator levels, whereas 76% of the samples scores 1-2 in the visual scale. In contrast, ATP measurements showed no differences before and after C&S procedures.

P1-122 Genomic Characterization and Engineering of *Salmonella* Enteritidis Phage to Construct a Nanoluc Reporter Phage

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❖ ***Developing Scientist Entrant***

Introduction: Within the ongoing challenges of *Salmonella* outbreaks, its rapid detection is an increasing necessity for public health. Reporter phage-based assays have emerged as a promising technology for the rapid and sensitive detection of *Salmonella*. Upon infection with reporter phages, target pathogens were identifiable by the expression of reporter genes from the engineered phage genome.

Purpose: The purpose of this study was to characterize and engineer the genome of *S.* Enteritidis (SE) phage to construct SE_{Nluc} reporter phage.

Methods: Once a whole genome sequencing of SE phage was conducted using an Illumina MiSeq system, its genome was annotated using BLASTP. The genome was genetically engineered to encode NanLuc luciferase (Nluc) by Gibson assembly. Following the electroporation of the assembled genome into SE cells, generating SE_{Nluc} phages within the host was confirmed by plaque assay and luminescent activity with a NanoGlo substrate. The specificity of the wild-type and the engineered phages was then assessed and compared using a plaque assay.

Results: The genome of SE phage consisted of 48,410 bp and 121 ORFs with 40.5% G+C content. PCR amplification revealed the successful insertion of Nluc into the downstream of capsid protein. In addition, seventeen recombinant plaques per transformation were obtained with SE_{Nluc} phages, enabling the detection of SE. Compared with a wild phage, SE infected with SE_{lacZ} phage produced significantly higher bioluminescent signals following the Nano-Glo addition (P<0.05). Furthermore, both wild and SE_{lacZ} phages could infect *S.* Enteritidis only.

Significance: The development of SE*lacZ* phage is promising to be extended as a rapid and sensitive detection method of SE in food.

P1-123 Transfer of *Salmonella enterica* Adhered and Embedded inside Biofilms on Plastic Surfaces to Dish Sponges during Simulated Dishwashing

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Introduction: *Salmonella enterica* exhibits the capability to form biofilms on inert surfaces, offering protection against disinfectants, antibiotics, and various stress factors, which facilitates its persistence and transmission.

Purpose: This project aimed to evaluate the transfer rate (TR) of *S. enterica* cells adhered and embedded within biofilms on plastic surfaces to a sponge, simulating a dishwashing scenario.

Methods: Three *S. enterica* strains was used, evaluating two scenarios: S1) cells adhered and S2) cells embedded inside biofilms on plastic surfaces. For S1, plastic surfaces were placed in a Petri dish with 10% chicken extract, native chicken microbiota (~5 log CFU/mL TPC), and S. enterica (~4 log CFU/mL). After incubation at 25°C for 4 hours, two washes with isotonic saline solution removed non-adherent cells. For S2, the same process was followed, with additional rinsing using 1% chicken extract and a subsequent 3-day incubation at 25°C and 97% relative humidity to encourage biofilm formation. Washing with a dish sponge (1.5x1.5 cm) and 2% soap solution followed, comprising two horizontal and two vertical washes. The TR of Salmonella enterica from surfaces to the sponge was calculated as [CFU_receiver/(CFU_donor+CFU_receiver)]*100.

Results: The scenario significantly influenced TR (p<0.05), with S1 exhibiting a higher TR (59.9 ± 16.6%) compared to S2 (5.1 ± 1.9%). Despite the low TR of S2, it is crucial to consider that the concentration on the plastic surface of adhered *S. enterica* strains (2.8 ± 0.5 log CFU/surface) was lower than in biofilms (5.7 ± 0.5 log CFU/surface). Consequently, a higher concentration of *S. enterica* was observed on sponges after dishwashing in surface with biofilms (4.4 ± 0.6 log CFU/surface) compared to surfaces with adhered cells (2.7 ± 0.4 log CFU/surface).

Significance: While TR is higher in S1, the increased *S. enterica* concentration transferred to the sponge in S2 highlights the impact of growth during biofilm formation.

P1-124 A Validation Study for the Tree Fruit Industry: the Use of Silver Dihydrogen Citrate (SDC) and Chlorine Dioxide Gas (ClO2) to Control *Escherichia coli* and *Listeria* on Picking Bags and Storage Bins

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks and recalls within the tree fruit industry are making producers re-evaluate appropriate cleaning and sanitation practices during harvesting. Without effective sanitation, bacteria can create niches and form biofilms. Validation of different sanitation methods can help producers develop strategies to reduce and mitigate risks.

Purpose: This study evaluates the efficacy of SDC and ClO_2 gas to control *E. coli* and *Listeria* in both sessile and biofilm forms on experimentally inoculated harvesting equipment at commercial orchards within the Midwest and Pacific Northwest (PNW) regions.

Methods: Rifampicin resistant *E. coli* and *Listeria* were grown for either 24-hours or 96-hours in 80ug/ml rifampicin TSB at 25± 2˚C on high density polyethylene plastic (HDPE), wood, or nylon coupons. Surfaces were allowed to dry for one hour and then exposed to ClO_2 (100 ppm) for 24-hours or SDC (4%) for two minutes. Coupons were swabbed, and remaining populations enumerated. Temperature and relative humidity conditions were noted. Experiments were conducted in triplicate and results significant at p< 0.05.

Results: ClO_2 was the most effective treatment (p<0.05) in controlling sessile *E. coli* and *Listeria* on HDPE and nylon in the Midwest and PNW. A lower level of inactivation was observed for biofilms grown on wood after ClO_2 treatment (p<0.05). In both regions, all biofilm populations on HDPE were reduced after exposure to SDC (p<0.05). SDC exposure did not reduce population of sessile *E. coli* on HDPE in the PNW, or on any form of *E. coli* on nylon in either region (p>0.05). Population reduction after SDC exposure was found on nylon surfaces inoculated with *Listeria* (p<0.05), except for biofilms in the PNW.

Significance: Validating sanitation procedures in various field conditions can help producers understand the most effective strategies to sanitize harvesting bins and picking bags.

Poster

P1-125 Survival of *Listeria monocytogenes* on Roller Brushes Made with Different Materials

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❖ ***Developing Scientist Entrant***

Introduction: Roller brushes play a significant role in microbial food safety as they come into direct contact with fresh produce. They are made with different materials to protect the surface integrity of fruits and vegetables. Unfortunately, the cleaning and sanitizing of roller brushes have been a challenge for the industry.

Purpose: This study aimed to investigate the survival of *Listeria monocytogenes* on roller brushes made with different materials.

Methods: Roller brush coupons (spiral pattern) made with 100% nylon and made with 50% horsehair and 50% polyenthylene were used. A five-strain cocktail of rifampin-resistant *Lm* were spot inoculated (100 µl/brush coupon) on brush surfaces. Inoculated roller brush coupons were then dried (24 hours) and stored at ambient temperature for 1 months. Samples were taken on Days 0, 1, 3, 7, 14, 21 and 28 to numerate the surviving *L. monocytogenes* on tryptic soy agar supplemented with 50µg/ml rifampicin.

Results: The initial *L. monocytogenes* inoculation levels on 100% nylon and 50% horsehair brush surfaces were 7.85±0.03 and 7.89±0.06 Log CFU/coupon respectively. After 24 hours drying, an approximately 0.65 Log CFU/coupon reduction was observed on both type of brushes. *L. monocytogenes* gradually declined during storage. At the end of Day 7, the survival of *L. monocytogenes* on both types of brushes were the same, at ca. 5.89 ± 0.03 Log CFU/coupon. After 28 days of storage, the population of *L. monocytogenes* declined by ~2.5 Log and ~2.8 Log on nylon and 50% brush coupons, respectively. Roller brush materials did not play significant impact on the survival of *L. monocytogenes* during ambient temperature drying and short-term storage (p>0.05).

Significance: *L. monocytogenes* contamination at packinghouses has been a food safety risk. Understanding the impact of roller brushes on the *L. monocytogenes* risk assessment and management is crucial for establishing efficient food safety plans.

P1-126 Implementation of Targeted Cleaning and Sanitation Directed by ATP Swabbing as a Part of Environmental Monitoring Program

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Introduction: ATP derived from food residues is used as an indicator to confirm cleaning, therefore the establishment of management standards and methods is drawing attention.

Purpose: Introducing the ATP program to a food manufacturing plant and verifying its effectiveness by comparing the detection of microorganisms.

Methods: From the selected Korea Citron Tea manufacturer, 64 test points in the highest-risk area were decided to evaluate the initial cleaning condition and any improvement through Clean-Trace® Luminometer with surface ATP Swab that can visualize the relative ATP level. The total test points were selected as 17 for P/F level and collected 14 times over 3 months, each level was evaluated using the mean value plus 3 σ from the collected ATP values from normal cleaning surface. Microbial changes were evaluated to confirm the effectiveness of ATP P/F level, simultaneously, by inoculating the swab sample to Petrifilm® twice with 1 mL each.

Results: Primary test points were set to 64 through the initial cleaning condition and finally set to 17 considering the degree of risk such as contamination proximity and physical distance. The P/F level was set as 75 RLU for nozzle that showed the highest ATP value, while hopper interior with the lowest value was selected as 50 RLU. When the nozzle, nozzle interior, steam supply device and cap feeder were tested in a total of 98 tests, 35 tests were confirmed as failure level, through periodic verification, each point was reduced to 25 to 40 RLU at last, which all meet the acceptance level. The microbiological results initially showed average coliform and aerobic count of 5 and 55 CFU/100cm^2, respectively but ultimately reached a level where microorganisms were rarely found.

Significance: Appropriate verification of cleaning through confirmation of ATP values can achieve improvement in microbial safety by reducing cross-contamination as part of environmental monitoring.

P1-127 Portable Assays for the Detection of Mycotoxins, Allergens and Sanitation Monitoring

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Introduction: Many food recalls are related to the presence of undeclared allergens and microorganisms in food products. To reduce these occurrences, portable diagnostic assay kits are available to quantitate mycotoxins, detect allergens and gluten in foods and on environmental surfaces and for sanitation monitoring.

Purpose: This presentation reviews several portable diagnostic kits that can detect sources of contamination in food and ingredients as well as on surfaces and clean in place (CIP) rinses. The use of these diagnostics tools for Hazard Analysis and Critical Control Points (HACCP) programs will be presented.

Methods: Mycotoxins and gluten were detected using lateral flow diagnostic (LFD) assays. Sanitation monitoring of surfaces was completed using a chemiluminescent assay to detect ATP and another assay to detect protein. ATP standard solutions at 100, 25.0, 12.5, 6.25, 3.12, and 0 femtomoles were measured with 10 replicates. Standard deviations (SD) were determined using the Microsoft Excel function STDEV.S. Assay methods and calculation of limit of detection (LOD) are described in detail in the body of the work.

Results: Gluten was detected at 10 ppm in spiked commodities and on wet and dry surfaces at 2.5 µg/100cm^2. Deoxynivalenol was quantitated in Dry Distillers Grains plus Solubles (DDGs) and mean results were within two SD of those determined by high performance liquid chromatography (HPLC). The chemiluminescent assay had a LOD of 6 femtomoles of ATP and was able to detect a 1:10,000 dilution of orange juice from surfaces. The protein assay detected 5 µg of BSA directly applied to the sampler, 100 µg of BSA on surfaces, and detected 1:10 dilutions of Greek yogurt and raw beef from surfaces.

Significance: Portable diagnostic kits evaluated in this work provided accurate, rapid, and sensitive results for detection of mycotoxins, gluten, proteins, and ATP. These methods can be used in facilities with minimal training as part of their HACCP plans to help maintain food safety.

P1-128 Development of Fluorescent Surrogate for Mimicking the Presence of Foodborne/Bacterial Pathogens and Promoting Sanitation

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Introduction: Curcumin is a diarylheptanoid belonging to a group of curcuminoids that displays green fluorescence under UV light. Fluorescent biomimetic particles (fBMPs) are food-grade, affordable, and have properties mimicking the surfaces of microbial spoilage and pathogenic organisms. Providing fBMPs as a visual indicator of sanitation will lead to enhanced cleaning practices and greater adherence to standardized SOPs.

Purpose: This study aims to develop fBMPs with surface properties that mimic bacterial surfaces for sanitation training and confirmation.

Methods: *E. faecium* (NRRL B -2354) is cultured and then centrifuged to obtain a pellet suspended in filter-sterilized deionized water to an OD600 of 0.9–1. The bacterial suspension was pipetted onto stainless steel coupons and placed in a desiccator. Bacterial charge and adhesion ability on stainless steel coupons were then measured. Curcumin fBMPs with Potato Protein 200 (PP200) were prepared, and their charge, size, and adhesion ability were measured and compared with those of *E. faecium.*

Results: Under the experimental conditions, the surface charge of *E. faecium* was negatively charged (-32.44 mV average). A solution containing 1% PP200, 50 mM NaCl, and corn oil was optimal for a similar charge (-22.8 ± 0.6) to E. *faecium*, as well as an oil-in-water emulsion with a mixture of 2% Tween 80 and PP200 that also displayed favorable, stable charge of -26.9±2.4 mV.

Poster

Significance: Curcumin-potato protein fBMPs demonstrated favorable stability and surface charge comparable to E. faecium, suggesting the potential of the fBMPs to serve as a visual surrogate that mimics the adhesion and removal behavior of bacteria for sanitation training applications.

P1-129 Systematic Comparison of Susceptibility of Seven Bacteria Genera to Blue Light Treatment

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial blue light (aBL; 405 nm) has been reported to reduce the viability of foodborne bacteria. However, there are limited reports on parallel comparisons of aBL susceptibility of multiple bacteria under the same conditions.

Purpose: This study was undertaken to evaluate the efficacy of aBL against foodborne pathogenic and spoilage bacteria under different experimental conditions.

Methods: Mixed strains of *Listeria, Salmonella, Escherichia, Pseudomonas, Cronobacter, Lactobacillus*, and *Staphylococcus* were inoculated in tryptic soy broth (TSB) and phosphate buffered saline (PBS), on stainless steel coupons as dry cells and biofilms, and on avocados and cherry tomatoes. Cell viability reductions were determined after exposure to 405 nm aBL at 4 °C and room temperature (RT). Total aBL dose for liquid, dry cells and biofilms, and fruits were 1,512, 1,739, and 1,944 J/cm^2, respectively. Microbiological testing was conducted by plating on standard complex media, followed by incubation at 37 °C for 24–48 h. ANOVA was used to determine significant differences (p<0.05).

Results: In PBS, the viability reduction of *Staphylococcus* and *Lactobacillus* was <3.5 Log CFU/mL but in TSB their viable counts decreased > 7 log CFU/mL (p<0.0001). The biofilm counts were reduced less than 4 log CFU/mL for all bacteria with the exception of *Staphylococcus* that was 4.6 log CFU/mL (p<0.05). Treatment of cells dried on avocados resulted in small reductions of *Cronobacter* and *Salmonella* (<1.5 log CFU/mL) at RT compared to other species. The viability of *Pseudomonas* was reduced more than 4.6 log CFU/mL after aBL exposure of tomatoes and avocados. The viability of *Listeria, Escherichia* and *Staphylococcus* on fruits were variable depending on the testing conditions.

Significance: These findings suggest that the susceptibility to aBL can vary markedly among bacterial species, in particular among environmental contaminant organisms.

P1-130 "Dry Steam" Treatments Result in Rapid Microbial Inactivation in a Narrow Radius Surrounding the Nozzle, Making Effective Manual Operation of Commercial-Scale Units Difficult for Human Users

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❖ ***Developing Scientist Entrant***

Introduction: Although bench-scale studies have shown that superheated steam is effective for microbial inactivation on surfaces, commercial-scale systems have not been evaluated.

Purpose: (1) To characterize the surface temperature gradients on stainless steel surfaces outward from the impingement point and (2) determine inactivation of *Enterococcus faecium* NRRL B-2354 during stationary and manual operation of a commercial-scale superheated steam unit.

Methods: The geometric center of stainless-steel surfaces (304, 2B, 30.5 x 30.5 cm^2) was treated with a stationary commercial-scale superheated steam unit (Bayzi Corporation, Cincinnati, OH) for 5 min at 397°C. The surface temperatures outward from the impingement point under varying surface thicknesses (0.05, 0.28, 0.48 cm), ambient temperatures (23.5, 12.8, 4°C), and nozzle-surface distance (2.5, 5.1, 7.6 cm) were measured with K-type thermocouples. *E. faecium* was dried down spot-inoculated (0, 2.3, 4.6 cm from the geometric center) on stainless steel surfaces and exposed (2, 5, 10 s) to stationary superheated steam. Human subjects (n=24) completed trainings about operation of superheated steam equipment and were asked to treat inoculated stainless-steel surfaces on two days. An ANOVA and Tukey tests were performed.

Results: Surface temperatures exceeded 300°C at the impingement point but decreased as surface thickness, nozzle-surface distance, and radial distance on the surface increased. Ambient temperature negatively impacted surface temperatures. Distance on the surface and time significantly impacted microbial inactivation (p<0.05) with the largest reduction of 9.60 $\pm$ 0.13 log CFU/cm^2 at the impingement point at 10 s. The microbial inactivation achieved by human subjects significantly increased with training interventions and experiential learning (p<0.05) but was not significantly influenced by prior experience with food safety or industry. The highest reductions achieved by human subjects were 3.56 $\pm$ 1.27 log CFU/cm^2.

Significance: Manual superheated steam sanitation equipment may be improved by expanding the treatment radius to increase ease of operation.

P1-131 Analyzing the Effectiveness of Chlorine-Based Sanitizer Spray in Removing Bacteria from Stainless Steel Surfaces with a Focus on Shear Stress

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Introduction: The effectiveness of chlorine-based sanitizer sprays on removing bacteria from surfaces can be influenced by the shear stress and the duration of contact.

Purpose: To quantitatively assess the bacterial removal of sanitizer spray on a stainless-steel surface using a commercial sprayer, we evaluated wall shear stress on the surfaces through computational fluid dynamics (CFD) modeling and experimentally examined the bacterial removal at specified locations on the surface with different shear stress exposures.

Methods: A commercial sprayer (SP-5L, Foamit, Grand Rapids, MI) was filled with 100 ppm sanitizer (XY-12), and the spray flow rate was 6 L/min. Stainless steel surfaces (2B finish, 34 x 34 cm^2) were spot inoculated with a cocktail of 3 *Listeria innocua* strains at three select locations: the center impingement location ('Impingement'), 5 cm to its left ('Left'), and 7.5 cm below it ('Down') to encompass variation in shear stress caused by the fan spray. The spray nozzle was positioned 28 cm away, perpendicular to the surface. A CFD model was developed based on flow properties and surface dimensions to analyze the shear stress exerted on the surface.

Results: Following a 3-sec sanitizer spray, the bacterial removal at the 'Impingement', 'Left', and 'Down' locations were 7.5, 5.7, and 0.4 log CFU/surface reduction, respectively. Comparatively, a 3-sec water treatment only resulted in reductions of 3.4, 3.2, and 0.4 log CFU/surface. At the 'Down' spot, extending the down-flow time of the sanitizer from 3 sec to 2 min resulted in an increased removal of 5.7 log CFU/surface. Additionally, incorporating a 2-min contact time after treatment enhanced the removal by 1.6 log CFU/surface. Lowered shear stress significantly reduced the effectiveness of microbial removal.

Significance: The developed model and experimental setup offer potentials for predicting the efficacy of environmental sanitizer sprays at various velocities, distances, and angles for future sanitation practices.

P1-132 Inactivation of *Geobacillus stearothermophilus* and *Clostridium sporogenes* Spore and Vegetative Cell Counts in Retort Packaged Food with Superheated Steam Sterilization

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❖ ***Developing Scientist Entrant***

Introduction: The demand for HMR (home meal replacement) food has increased, especially for long-time storage. Thus, the decontamination technology is important for the products because the bacterial endospores need to be destroyed.

Purpose: The objective of this study was to evaluate the antibacterial effect of superheated steam sterilization (SHS) on spores and vegetative cells of *Geobacillus stearothermophilus* and *Clostridium sporogenes* (surrogate for *Clostridium botulinum*) in kimchi stew with pork and *Seonji-Haejangguk* (beef blood soup) packaged in retort pouches.

Methods: One milliliter aliquots containing spores and vegetative cells of *G. stearothermophilus* and *C. sporogenes* were inoculated in 250g of kimchi stew and *Seonji-Haejangguk* in retort pouches, which were then heat-sealed. These packaged samples were treated with SHS at 140°C for 1 sec, 1 min, and 3 min or thermal sterilization at 120°C for 1 min, 3 min, and 5 min. Spores and vegetative cell counts in the sterilized samples were enumerated on Nutrients Agar and Reinforced Clostridial Agar for *G. stearothermophilus* and *C. sporogenes*, respectively.

Results: In kimchi stew and *Seonji-Haejangguk* with SHS for more than 1 min, spore and vegetative cell counts of *G. stearothermophilus* and *C. sporogenes* were below the detection limit (0.5 Log CFU/g). After the thermal sterilization for 4 min, spore and vegetative cell counts of *G. stearothermophilus* and *C. sporogenes* were below the detection limit in all samples.

Significance: These results indicate that SHS at 140°C for 1 min may reduce bacterial vegetative cell and spore counts below the detection limit, which is a similar result to the thermal sterilization at 120oC for 4 min. However, this result might be varied by food ingredients and cooking methods. Thus, further studies are necessary.

P1-133 Comparative Antimicrobial and Anti-Biofilm Activities of Postbiotics against Bovine Mastitis Pathogens

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Introduction: Bovine mastitis, caused by pathogens including *Staphylococcus aureus, Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Escherichia coli*, poses a significant challenge to the dairy industry.

Purpose: This study aimed to investigate the underlying mechanisms of the anti-microbial and anti-biofilm activities of postbiotics derived from lactic acid bacteria against bovine mastitis pathogens.

Methods: The mechanisms of anti-microbial and anti-biofilm activity were demonstrated by analyzing biochemical analysis, organic acid quantification of postbiotics, and modulation in biofilm-related phenotypes and genotypes, such as hydrophobicity, auto-aggregation, and exopolysaccharide (EPS) production of pathogens.

Results: Kefir-derived *Lentilactobacillus kefiri* LK1 produced significantly more lactic acid than normal raw milk-derived *Enterococcus faecium* EFM2, which was attributed to differences in biochemical activities. At an optimum concentration of 25%, both *L. kefiri* LK1 (POS_LK1) and *E. faecium* EFM2 postbiotics (POS_EFM2) exhibited anti-microbial and anti-biofilm activities by modulating hydrophobicity, auto-aggregation, and EPS production phenotypes and genotypes of bovine mastitis pathogens. POS_LK1 treatment reduced auto-aggregation and EPS production by *E. faecalis* and downregulated wspA and pelA expression in *P. aeruginosa* compared to those in POS_EFM2.

Significance: Our results contribute to sustainable livestock farming based on novel development strategies for alternative or complementary treatment in bovine mastitis management.

P1-134 Biofilm Development and Removal on/from HDPE Coupons of Various Colors Using Selected Sanitizing Treatments

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❖ ***Developing Scientist Entrant***

Introduction: Packing facilities for fresh blueberries have developed various protocols to clean/sanitize harvest containers to prevent cross-contamination between berries and their surfaces. However, it is unknown whether these cleaning/sanitizing practices could effectively remove biofilms formed by microorganisms from blueberry production/packing environments.

Purpose: This study investigated the effectiveness of some cleaning and sanitization practices in removing biofilms formed on coupons of high-density polyethylene (HDPE), a material of blueberry harvest containers. It also assessed whether the color of HDPE had any effect on the formation and removal of biofilms.

Methods: Three inoculums of fecal coliforms isolated from fresh market blueberry harvest/packing environments were used to develop biofilms on HDPE coupons with various colors (n=7). Coupons of selected colors (yellow and orange) with developed biofilms were treated with sterile water, 100 ppm sodium hypochlorite, and 2% liquid dish soap, respectively with or without a 5-min soaking, manually or using a benchtop wash machine for 1 min. The experiment was replicated twice, and each treatment was duplicated. Biofilms or their residues were quantified using the crystal violet binding assay. Data collected were analyzed using the Analysis of Variant of the SAS.

Results: Treatments with soaking and using the bench top wash machine removed significantly ($P \leq 0.05$) more biofilms from coupons than those without soaking and manual washing. Sodium hypochlorite-treated coupons had significantly more biofilm residues than dish soap-treated coupons, while no differences ($P > 0.05$) in biofilm residues were observed between these two types of coupons and water-treated coupons. Biofilm residues on the orange coupons were significantly higher than on the yellow coupons, although coupon color did not have a significant impact on biofilm formation.

Significance: The concentration of sanitizer and detergent used by some packing facilities may not be adequate to eradicate biofilms from berry-contact surfaces. The study reveals the challenges of maintaining the hygiene of blueberry harvest containers.

P1-135 An Immunoblot Culture Procedure for the Isolation of *Salmonella* Enteritidis from Poultry Environmental Samples

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Introduction: Rapid methods for the detection of *Salmonella* Enteritidis (SE) from poultry environmental samples are widely used to identify potentially contaminated flocks for de-population and prevent contaminated eggs from reaching the market. However, culture methods used for confirmation of rapid screening results lack specificity for *S.* Enteritidis. Since the vast majority of samples contain more than one serovar, the selection of few colonies for *S.* Enteritidis confirmation presents a problem in that the fittest and abundant serovars are selected, potentially missing *S.* Enteritidis. An immunoblot culture

procedure targeting Group D1 *Salmonella* was developed to improve *S.* Enteritidis isolation.

Methods: Following incubation on selective agars, nitrocellulose membranes were overlaid onto agar surfaces to allow for colony transfer. Membranes were then sequentially exposed to rabbit anti-*Salmonella* O Antiserum Factor 9, followed by Alkaline Phosphatase-Conjugated Goat Anti-Rabbit IgG rinsing with a wash buffer before each step. The membranes were then incubated in BCIP/NBT substrate in order to visualize the location of suspect colonies appearing as intense purple dots. Colonies were matched to the agar plates and confirmed by a *S.* Enteritidis-specific PCR.

Results: The immunoblot assay achieved 100% specificity and 100% sensitivity from analysis of Group D1 *Salmonella* strains (n=30, among which 21 were SE isolates) and 30 exclusivity strains. When applied to samples inoculated with *S.* Enteritidis and *S.* Infantis as a competitor or with naturally occurring *Salmonella* from farm environments, the assay facilitated the isolation of *S.* Enteritidis. In contrast, artificially introduced *S.* Enteritidis was unable to be isolated through typical culture methods in the presence of competing *Salmonella*.

Significance: This immunoblot procedure is capable of distinguishing Group D1 *Salmonella* among other competing *Salmonella* strains and can become a helpful tool in the confirmation of viable *S.* Enteritidis from poultry environmental samples where traditional culture methods continue to be the gold standard for regulatory agencies and other stakeholders.

P1-136 Development of a Method to Allow Biofilm Kill Claims for Disinfectants Used in Food Production

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Introduction: The US Environmental Protection Agency (EPA) has established criteria for disinfectant claims against biofilm on hard, non-porous surfaces using *Pseudomonas aeruginosa* and *Staphylococcus aureus.* However, the agency has determined these organisms have limited relevance to food production and is requiring data using an organism considered more relevant, *Listeria monocytogenes*. The EPA has accepted at least one protocol, but the method lacks reproducibility and generates a low-density biofilm that is easy to kill.

Purpose: Develop a more robust and reproducible method to be used for biofilm kill claims against *L. monocytogenes*.

Methods: The CDC biofilm reactor was used in the EPA accepted protocol. Modifications to this protocol were explored including an increase in media concentration and flow rate. Alternatively, a method that involves growing *L. monocytogenes* with *P. aeruginosa* in a CDC reactor was investigated. Biofilm cell density was determined using standard plate count methods with confirmation of *L. monocytogenes* based on biochemical testing and selective media growth. Fluorescent microscopy was used to visualize biofilm architecture. The Single Tube Method was used to determine biofilm susceptibility to peracetic acid (PAA).

Results: Modification of the current EPA accepted protocol increased the mean log density on control carriers from 6.70 ± 0.28 to 7.42 ± 0.27. Nevertheless, it was relatively easy to achieve a 6-log reduction with PAA (100 ppm) against this biofilm compared to a standard *P. aeruginosa* biofilm (> 700 ppm PAA). By combining *L. monocytogenes* with *P. aeruginosa*, a higher log density of *L. monocytogenes* was obtained (8.46 ± 0.22) and a higher level of PAA (300-500 ppm) was required to achieve a 6-log reduction.

Significance: Minor modifications can increase the reproducibility and biofilm log density of the current EPA accepted protocol, but a more significant improvement can be made by growing *Listeria* in a co-biofilm with *P. aeruginosa*.

P1-137 The Efficacy of O3-Nanobubble Solutions against *Listeria monocytogenes* Biofilms on Food-Contact Surfaces

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Introduction: *Listeria monocytogenes* contamination on food-contact surfaces has been identified as significant cause of numerous food outbreaks and recalls, emphasizing the crucial need for efficient surface sanitization. Ozone is a strong oxidizer and potent antimicrobial, and the ozone nanobubble (ONB) solution represents a novel form of ozone with an extended shelf-life. However, its efficacy against *L. monocytogenes* biofilm is unknown.

Purpose*:* To examine the efficacy of ONB against *L. monocytogenes* biofilms on different food contact surfaces.

Methods*:* The 2- and 7-day-old *L. monocytogenes* biofilms were formed on stainless steel, polyester, and rubber surfaces. Then, these biofilms were subjected to ONB solution treatments at various conditions. The surviving *L. monocytogenes* cells on each coupon was detached and enumerated.

Results*:* ONB solution exhibited similar efficacy against *L. monocytogenes* biofilms on different food contact surfaces, regardless of the biofilm age. There were 0.9 - 1.4 log CFU/coupon reductions of *L. monocytogenes* in 7-day-old biofilms on all surfaces tested after 1 min treatment of ONB at 4 ppm. Extending treatment time from 1 to 10 min did not improve efficacy. However, increasing the volume of ONB during treatment significantly improved its efficacy against *L. monocytogenes* biofilms on stainless steel surface. In addition, worn stainless steel did not impact the efficacy of ONB, whereas defects on polyester and rubber surfaces significantly diminished the efficacy of ONB.

Significance: Data provide valuable insight into the antimicrobial effectiveness of ONB against *L. monocytogenes* biofilms on food contact surfaces.

P1-138 Inactivation of *Listeria* biofilms by Hurdle Treatments of Peroxyacetic Acid and Saturated Steam

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Introduction: Contaminations of food contact surfaces by *Listeria monocytogenes* have been implicated multiple outbreaks and food recalls. The presence of *L. monocytogenes* biofilms on equipment surfaces poses a potential source of contamination. It is crucial to implement effective surface sanitization to eliminate *L. monocytogenes* contamination on food-contact surfaces.

Purpose*:* To evaluate the efficacies of hurdle treatments combining peroxyacetic acid (PAA) and saturated steam against *Listeria* biofilms on common food-contact surfaces under various surface conditions.

Methods*:* The 7-day-old *L. innocua* biofilms were formed on stainless steel (SS), polyester (PET), and rubber surfaces under different conditions. Then, these biofilms were subjected to PAA and steam treatments, alone or in combination. The surviving cells were then detached from each coupon and enumerated.

Results*:* The treatment combining PAA at 40-80 ppm for 1 min and steam (100°C, 6 s) treatment caused > 6 $\log_{10}$CFU/coupon reduction of *L. innocua* on SS and PET surfaces and ~ 5 log CFU/coupon on rubber surfaces. The efficacies of the PAA and steam hurdle treatment was compromised by apple juice conditioning and surface defects. However, the application of 40 ppm PAA 1min followed by 6 s steam treatment still led to > 5 log CFU/coupon reductions of *L. innocua* on worn SS and PET soiled with apple juice, and ~ 4.5 $\log_{10}$ CFU/coupon *L. innocua* reduction on the worn and soiled rubber surface.

Significance: This study suggested that PAA treatments followed by a quick saturated steam exposure is viable strategy for surface disinfection.

P1-139 Electrostatic Powder Coating Inoculation Methodology for Dry Sanitation Experimentation

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❖ ***Developing Scientist Entrant***

Introduction: Modeling a replicable pathogen cross contamination event in dry processing environments remains challenging. However, simulating electrostatic powder accrual on equipment surfaces may provide an improved representation of cross contamination for dry cleaning and sanitation experimentation.

Purpose: The purpose of this study was to develop a standardized dry inoculation methodology using an electrostatic coating process for dry sanitation

experimentation.

Methods: A wheat berry mixture (80/20 ratio of hard winter red and soft white wheat, 1000 g) was inoculated using a six-strain *Salmonella* cocktail, to model contamination of raw agricultural products, and equilibrated to ~0.45 a_w in a controlled-humidity chamber for 48 ± 2 h. The wheat berries were fabricated into all-purpose flour using a KitchenAid® metal grain mill followed by mechanical sieving. The fabricated flour (~8.25 log CFU/g) was applied to #304 and #316L stainless steel coupons (32.18 cm^2) using an electrostatic powder coating gun. Samples (n = 48) were either coated (a positive control) or coated then brushed to visibly clean using a soft bristled brush. *Salmonella* was recovered by hand-massaging bagged coupons with buffered peptone water, which was serially diluted and enumerated on Modified Tryptic Soy Agar.

Results: Within each stainless-steel grade, there were significant differences in remaining *Salmonella* populations between coated and brushed coupons ($p < 0.0001$). Coated coupons had 5.45 ± 0.37 log CFU/cm^2 (#304) and 5.65 ± 0.14 log CFU/cm^2 (#316L) (mean ± 95% CI). Brush-cleaned coupons had 3.92 ± 0.36 log CFU/cm^2 (#304) and 4.10 ± 0.39 log CFU/cm^2 (#316L). Brush-cleaning reduced *Salmonella* populations on stainless steel surfaces by ~1.5 log.

Significance: The dry powder coating inoculation methodology was consistent and replicable at pilot scale. Brushing of contaminated surfaces until they appear visibly clean does not ensure microbial cleanliness.

P1-140 Antimicrobial Efficacy of Dry Sanitizers under Ambient Conditions

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Introduction: Dry sanitizers are applied to the floors of food processing facilities to control microbial pathogens and prevent their spread between facility locations. The products have been shown to be readily activated and effective in the presence of water, but limited data exists with regards to efficacy in drier environments.

Purpose: Assess the antimicrobial efficacy of two dry sanitizer products with different active compositions against food pathogens under ambient conditions at various relative humidities.

Methods: Antimicrobial activity was determined by two methods. The first involved drying bacteria (*Listeria monocytogenes* or *Cronobacter sakazakii*) on stainless steel carriers with a soil load, applying powder to the carriers, followed by incubation at 35% and 70% relative humidity (RH). After 4h and 24h, the number of bacteria remaining was determined following neutralization and serial dilution plating. In the second method, powder was applied to a Petri dish which was then incubated for 72h at 35% and 70% RH. Bacterial contaminated boot stamps were then walked through the powder and additional dishes. The number of bacteria in those dishes was determined after 5min as described above. Log reduction (LR) values were determined by comparison to bacterial counts from surfaces with no powder.

Results: With the first method both products demonstrated efficacy against dried bacteria under ambient moisture conditions. The LR values ranged from 0.52 - 4.01 for *L. monocytogenes*, and 0.85 - 3.46 for *C. sakazakii*. In general, the LR values increased with RH and contact time and were only slightly impacted by soil type. With the second method, both products were effective in reducing cross contamination after 72h conditioning. The LR values ranged from 1.88 - 6.03 for *L. monocytogenes*, and 2.35 - 6.01 for *C. sakazakii*, and appeared to be independent of the RH storage conditions.

Significance: The results show dry sanitizers can be effective under ambient moisture conditions. Furthermore, they can reduce cross contamination in dry environments.

P1-141 Mobile Flow Cytometer for In-Situ Measurement of Microbial Contamination on Food Contact Surfaces in Restaurants

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Introduction: The occurrence of cross-contamination in food contact surfaces increases the risk of foodborne diseases incidence, particularly when the premises are not being monitored. To determine the cleanliness level, microbial detection and counting is of great importance for decision making of effective pre-operational action to improve food handling and premises rating. Routine method to detect microbes require laboratory analysis that is time consuming.

Purpose: We explore the capability of mobile flow cytometer device namely as CytoQuant® as one of the latest technology for measuring in situ microbial contamination comparing with Total Plate Count (TPC) analysis. This device is employing an impedance-based detection system which commonly associated with drinking water hygiene.

Method: The cross-sectional study using the flow cytometry and TPC involved 30 food contact surfaces from six (n=6) selected restaurants with long operating hours for a duration of 3 months. Findings showed that the flow cytometry was able to detect the presence of microbial contamination within 30 seconds without pre-treatment and incubation for each contact surface of food taken by the swab. The results also showed intact cell and particles readings on the contact surfaces of the food studied such as cutting boards, knives, clips food, plates and spoons. There were no significant differences ($p > 0.05$) between intact cell readings using the flow cytometry and the TPC analysis (t = 440.00).

Significance: In summary, using CytoQuant® on site to monitor the cleanliness level of food contact surfaces may prevent the risk of foodborne diseases as it is easy and robust as compared to TPC analysis. The prompt microbial contamination detection will help the enforcement to take further steps for advising the food handlers to take precautions in preparing and serving food to consumers.

P1-142 Phage Biocontrol of *L. monocytogenes* Attached to Food Contact Surfaces

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Introduction: *Listeria monocytogenes* is a major foodborne threat with high mortality and persistence in food processing. It forms biofilms on various food and non-food contact surfaces. While current methods use cleaning agents, phage-based biocontrol is gaining recognition for comprehensive food safety enhancement and bio-sanitation on contact surfaces.

Purpose: Investigate the efficacy of single *Listeria* phage LP-125 at reducing *L. monocytogenes* biofilms formed on different food contact surfaces (FCS).

Methods: Stainless steel (SS), ethylene propylene diene terpolymer (EPDM), High-Density Polyethylene (HDPE), and polystyrene (PE) surfaces were used in this study. FCS were cut into coupons and inoculated with *L. monocytogenes* 10403s strain at 2.5 x 10^7 CFUs of each well in a 24 well plate at 22 ºC for 24 h. After PBS washing, each FCS was treated with LP-125 phage at total concentration of 2.5 x 10^9 PFU's for 1 h or 24 h. Bacterial colonies were enumerated after vortexing the coupon in PBS. Additionally, control experiments and the rate of phage adsorption was determined on biofilm cells from FCS.

Results: Overall, there was 1.3–1.6 and 1.4–1.8 log CFU/coupon, reduction of *L. monocytogenes* cells on SS, and EPDM surfaces after treatment with a total of 2.5 x 10^9 PFU's of *Listeria* phage LP-125 at 25 °C. The bacterial reduction differed by plastic surface, with a 1.5 log CFU decrease on HDPE. No significant difference was observed between bacterial counts on a 96 well plate type and a slightly lower reduction of ~0.9 log CFU per well on a 24 well plate. Control experiments demonstrated that residual phages did not impact the reported log reductions. LP-125 phage adsorption was observed at similar levels under all conditions tested.

Significance: A single phage was able to significantly reduce attached *L. monocytogenes* biofilms and the reduction was influenced by type of surface used.

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P1-143 Food Safety Training in the Western Region of the United States from 2018 to 2021: Knowledge Gain and Teaching Ability Evaluations Identify Future Training Needs

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Introduction: Ongoing evaluation of food safety trainings is essential to ensuring the food industry has access to effective food safety education to support their compliance with Food Safety Modernization Act regulations. The Western Regional Center to Enhance Food Safety (WRCEFS) coordinates and collates training data from across the Western United States to evaluate training effectiveness and identify future training needs.

Purpose: Evaluate perceived and measured knowledge gain across standardized food safety trainings, including trainers' perceived ability to teach standardized curricula.

Methods: Knowledge gain was assessed using pre- and post-training tests (paired t-test, $p<0.05$) for participants in 108 Produce Safety Alliance (PSA) Grower Trainings (GT; 57 in-person; 51 remote) and 11 Food Safety Preventive Controls Alliance Preventive Controls for Human Food (PCHF) courses (5 in-person; 6 remote) in the Western region from 2018 to 2021. Additional feedback was collected by PSA from 10 Train-the-Trainer courses (TTT; 181 trainees) and 145 GT (2,000 participants) courses about perceived knowledge (GT and TTT) and ability to teach standardized curricula (TTT).

Results: Completion of the standardized PSA-GT and PCHF courses significantly increased participant knowledge (t=-27.27, $p<0.001$ and t= -4.88, $p<0.001$, respectively). Knowledge increase did not differ between in-person and remote courses. In both GT and TTT courses, 98% of attendees reported an increase in perceived knowledge. A majority (99/181) of PSA-TTT trainees felt they were able to deliver the PSA curriculum. Over 20% (39/181) of PSA-TTT trainees reported low confidence in teaching at least one module. These trainees had low confidence in teaching worker health and hygiene, production and post-harvest agricultural water, and farm food safety plan modules.

Significance: Participant evaluation data demonstrated effectiveness of the standardized PSA and PCHF curriculum to increase knowledge gain. PSA trainer evaluations provided helpful insight to identify content areas to support the ongoing development and effectiveness of trainers within the Western Region.

P1-144 Analysis of Cleaning and Sanitizing Methods to Reduce *Listeria* and *Salmonella* on Diverse Produce Contact Surfaces

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Introduction: Cleaning and sanitizing practices vastly differ across produce operations. Demonstrating the relative effectiveness of diverse practices can encourage growers to improve their cleaning and sanitizing programs and reduce the likelihood of expanded recalls.

Purpose: Evaluate the efficacy of cleaning and/or sanitizing methods on different produce contact surfaces contaminated with *Listeria* spp. or *Salmonella*.

Methods: Coupons (2.5 cm diameter; stainless steel [SS], high-density polyethylene [HDPE], plywood, or polyester-nylon) were inoculated with a five-strain cocktail of *Listeria* spp. or *Salmonella* with cabbage juice, inverted and incubated on tryptic soy agar with yeast extract (TSAYE; 37°C, 24 h). Coupons (9-10 log CFU/coupon) were rinsed only (RO), multi-step cleaned (MSC), sanitized only (SO; peroxyacetic acid [PAA], HClO, quaternary ammonium compounds [QAC]), or cleaned and sanitized (MSC+S). Half of the coupons were dried (15-60 min). Treated coupons were transferred to Dey/Engley broth with glass beads (1 g), vortexed and enumerated on Harlequin- or Hektoen Enteric-overlaid TSAYE.

Results: RO and MSC removed the least amount of pathogens from surfaces (ave. 0.4-3.5 log reduction). Sanitizer use (with/without MSC) significantly increased pathogen inactivation compared to MSC ($p<0.0001$). Sanitizer efficacies differed, PAA > HClO = QAC, regardless of prior cleaning status, and were improved with drying ($p<0.0001$). Targeted >5-log reductions were achieved consistently on SS with MSC+S (98%; 71/72). Treating with PAA (no MSC) was also effective on SS (92%; 11/12). HDPE was consistently sanitized using MSC+PAA (100%; 24/24) and MSC+HClO (92%; 22/24), but not MSC+QAC (67%; 16/24). Tested methods did not achieve a 5-log reduction on the polyester-nylon or plywood surfaces.

Significance: MSC+S effectively reduced *Listeria* and *Salmonella* on non-porous surfaces but did not achieve the targeted reduction on porous surfaces. Less ideal rinsing, cleaning or sanitizing procedures led to lower pathogen reductions. These data provide valuable information for the produce industry to make data-driven decisions for their cleaning and sanitizing practices.

P1-146 Synergistic Efficacy of Lactic Acid and UV-C in the Inactivation of *Listeria monocytogenes* on Food Contact Surface Materials

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Introduction: The control of *Listeria monocytogenes* within food processing environments remains a constant challenge. The consistent trend in *Listeria*-associated outbreaks coupled with a particularly high fatality rate justifies an urgent need to investigate alternative sanitation approaches suitable for organic and conventional operations.

Purpose: To assess the inactivation of *Listeria* monocytogenes on common food contact surface materials following exposure to lactic acid (2% v/v) and/or ultraviolet light (UV-C).

Methods: Five commonly used food contact materials were selected: stainless steel, Polyvinyl Chloride (PVC), High-Density Polyethylene (HDPE), Polytetrafluoroethylene (PTFE), and nylon. Coupons were dipped into a mixture of soil, water, and blueberries and air-dried overnight. Coupons were spot inoculated with 100 µL of *L. monocytogenes* cocktail (four produce-outbreak strains) and air-dried for 1 h. Antimicrobial treatments were applied from 1 to 5 min using a chamber equipped with a 254 nm UV-C lamp and a disinfectant spray system. Three replicates of four experimental treatments, including lactic acid (LA) and UV-C individually and in concert (LAUV), were investigated alongside negative (water-only) and positive controls. Coupons were neutralized in 30 mL Dey/Engley broth and 0.2 % Tween 80, vortexed, and spiral plated onto Tryptic Soy Agar and Modified Oxford Agar. Plates were incubated at 37 °C and enumerated after 48 h.

Results: Initial populations averaged 7.9 log CFU/coupon across materials. The highest reductions were observed on coupons treated with LAUV and LA, followed by water and UV treatments. The effects of sanitizer and sanitizer:material, but not material type, were significant ($p<0.05$). The most significant reduction, approximately 6 log CFU/coupon, was observed following the combined application of lactic acid and UV on HDPE for 1 min. However, no significant differences ($p>0.05$) were observed between LA and LAUV across materials.

Significance: Such targeted treatments present promising results in addressing *Listeria* persistence on food contact surfaces.

P1-147 Inactivation of *Salmonella enterica* and *E. coli* O157:H7 on Dry Stainless-Steel Surfaces by Ultra-High Irradiance Blue (405 nm) Light

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Introduction: The control of enteric pathogen contamination in dry operations represents a significant challenge due to their tolerance to desiccation stress and enhanced thermal resistance. Blue light is emerging as a safer alternative to UV irradiation for surface decontamination.

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Purpose: To assess the efficacy of ultra-high irradiance (UHI) blue (405 nm) LED treatments against *Salmonella* spp. and *E. coli* O157:H7 dry cells on clean and soiled stainless steel (SS) surfaces.

Methods: Sterile SS coupons (n=12) were spot-inoculated (70 µL) with a 5-strain cocktail of *S. enterica* and *E. coli* O157:H7 suspended in water or in a flour slurry (0.25 g/mL) to emulate the presence of organic soiling. Inoculated coupons were allowed to air dry for 3 h and subjected to blue light irradiation (842 mW/cm^2) at energy doses ranging from 221 to 1106 J/cm^2 (4−22 min). To determine the bactericidal mechanisms of blue light, the intracellular concentration of reactive oxygen species (ROS) and the temperature changes on SS surfaces were also measured.

Results: The treatment energy dose had a significant effect (p<0.05) on microbial inactivation levels. On clean SS surfaces, the reduction in *Salmonella* and *E. coli* counts ranged from 0.8−6.1 and 2.0−9.2 log CFU/cm^2, respectively. Blue LED treatments triggered a significant generation of ROS within bacterial cells (p<0.05), as well as a substantial temperature increase in SS surfaces. However, in the presence of organic matter, the oxidative stress in bacterial cells declined significantly (p<0.05), and treatments with higher energy doses (>700 J/cm^2) were required to uphold the antimicrobial effectiveness. The mechanism of the bactericidal effect of UHI blue LED treatments is likely to be a combination of photothermal and photochemical effects.

Significance: These findings indicate that LEDs emitting UHI blue light could represent a novel cost- and time-effective alternative for controlling microbial contamination in dry food processing environments.

P1-148 Sanitation Interventions for Reducing *Salmonella* on Cordura and Canvas Harvest Bags

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❖ ***Developing Scientist Entrant***

Introduction: Food contact surfaces, including harvest bags, should be cleaned, and sanitized to prevent potential foodborne contamination events. Recommendations for sanitation best practices of harvest bags are lacking.

Purpose: The objective of this study was to assess the efficacy of wet (chlorine, peroxyacetic acid, and water) and dry (isopropyl alcohol with quaternary ammonium compounds, and steam) based sanitizers on the reduction of *Salmonella* on harvest bag material-types (canvas and cordura).

Methods: Material-types were cut to 5x5cm coupons and inoculated with a 5-strain cocktail of *Salmonella* at ~7.4 ± 0.1 log CFU/coupon. The surfaces were air-dried in a biosafety cabinet for 1.5h until visibly dry. Inoculated surfaces were treated with chlorine (200 ppm), peroxyacetic acid (PAA; 200 ppm), isopropyl alcohol with quaternary ammonium compounds (IPAQuats; ready-to-use), steam and water. Sanitizers were applied following manufacturer's instructions for a 1-min contact time. Surfaces were subject to rub-shake-rub for 60s in 20mL of D/E neutralizing broth + 1% Tween80 and plating on selective (Xylose Lysine Deoxycholate-RP) and non-selective (Tryptic Soy Agar-RP) media. Duplicate experiments were conducted with five replicates per treatment combination (n=10/treatment combination; n = 50 total). Significant differences (p≤0.05) were evaluated by Tukey's HSD test in R-Studio (version 4.3.1).

Results: *Salmonella* reduction was significantly higher on cordura than on canvas when treated with chlorine, IPAQuats, steam and water (p<0.01). No significant differences in *Salmonella* reduction were observed between material-types when treated with PAA (P=0.13). The highest reductions of *Salmonella* by 4.6±1.0 and 5.9±0.6 log CFU/coupon were achieved with IPAQuats on canvas and cordura material-types, respectively. When surfaces were treated with PAA, *Salmonella* concentrations reduced by 3.7±0.8 and 3.2±1.1 log CFU on canvas and cordura, respectively. With chlorine, steam and water, reductions were ≤2 log CFU/coupon.

Significance: Sanitizer efficacy differed by material-type. Both wet and dry based sanitizers were effective against *Salmonella* when used correctly. IPAQuats were the most effective treatment for sanitizing canvas and cordura harvest bags.

P1-149 Impact of Brining on the Survival of Shiga-Toxigenic *Escherichia coli*, *Vibrio* spp. *Salmonella* spp. and *Listeria monocytogenes* on Inoculated Sugar Kelp (*Saccharina latissima*) During Refrigerated and Ambient Storage

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❖ ***Developing Scientist Entrant***

Introduction: The seaweed industry has several challenges including limited harvesting time and the short shelf life of fresh seaweed. One preservative method to extend the shelf life of sugar kelp is brining. However, studies on the impact of brining on pathogen survival in seaweed are limited.

Purpose: Therefore, the aim of this study was to investigate the impact of brining on the survival of pathogens inoculated on brined sugar kelp subjected to different temperatures during storage.

Methods: Fresh sugar kelp was inoculated (6.0 log CFU/g) separately with a cocktail of two strains of Shiga toxigenic *E. coli* (STEC) and *L. monocytogenes*, and two species and serovars of *Vibrio* and *Salmonella*, respectively. Inoculated samples were brined (40% weight/volume NaCl) to achieve a target water activity of 0.77 or less. Brined samples were stored at refrigerated and ambient temperatures for up to 12 weeks. Microbiological analyses were performed weekly to evaluate the survival of pathogens in brined kelp samples stored at ambient or refrigerated temperatures. A Multi-way ANOVA (p<0.05) was used to assess the effects of brining on pathogens' population during storage.

Results: The population of STEC, *Vibrio*, *Salmonella*, and *Listeria* was significantly reduced by 2.20, 2.21, 1.57, and 2.07 log CFU/g, respectively, after treatment compared with control. STEC and *Vibrio* count in stored brined kelp were <1.0 CFU/g after week 4 and were not detected in samples stored at refrigerated temperature after week 7. *Salmonella* was not detected in brined samples stored at refrigerated and ambient temperatures after weeks 10 and 4, respectively. *Listeria* survived in stored brined kelp for up to 10 weeks. Storage duration and temperatures showed significant interactions in the case of Salmonella only.

Significance: Brining may have a positive impact on pathogens' reduction in sugar kelp; however, additional steps should be taken to reduce microbial contamination levels to ensure safety.

P1-150 Pathogenicity Prediction of *Vibrio parahaemolyticus* by Using Pangenome Data with High Performance Machine Learning Algorithms

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❖ ***Developing Scientist Entrant***

Introduction: *Vibrio parahaemolyticus* has been a significant food safety concern due to its prevalence in seafood, causing considerable public health challenges and economic losses globally. Novel, sophisticated, and artificial intelligence-driven analytical tools that warrant more rapid and accurate virulence detection of *V. parahaemolyticus* can better inform risk assessment and management.

Purpose*:* This study aims to develop and validate new and precise classification models that can accurately differentiate clinical pathogenic *V. parahaemolyticus* from non-clinical/non-pathogenic *V. parahaemolyticus* isolates based on pangenome data.

Methods*:* The record of 2069 complete *V. parahaemolyticus* genome assemblies were found in the National Center for Biotechnology Information GeneBank. Among them, 1981 genome assemblies (including 872 clinical and 1109 non-clinical isolates) were able to be downloaded. Isolation source-based labels were obtained from the metadata and the pangenome was constructed with Roary using genome annotated files from Prokka. Four different sizes

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of pangenome matrix (core, soft-core, shell, and whole pangenome) were constructed and used as the database for *V. parahaemolyticus* pathogenicity prediction. Basic machine learning (ML) models including Gaussian mixture model, Support Vector Machine, Random Forest (RF), and K-Nearest Neighbors were applied using scikit-learn. Advanced deep learning (DL) models including multi-layer perceptron (MLP) and convolutional neural network (CNN) were applied using PyTorch. All models were 10-fold cross-validated.

***Results**:* Among basic ML models, RF showed the best performance in *V. parahaemolyticus* pathogenicity prediction (average accuracy ca. 84%). The performance of basic ML models got enhanced as the number of input gene features in pangenome matrix increased. For deep learning models, the performance of CNN (average accuracy ca. 92%) surpassed that of MLP (average accuracy ca. 88%), highlighting its capability to identify complex patterns in pangenomic data.

Significance: This study compared the efficacy of different ML and DL models in predicting the pathogenicity of *V. parahaemolyticus*, providing an innovative tool for more accurate and rapid pathogenicity assessments.

P1-151 Identification of *Vibrio* in Oyster with FISH Technique

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Introduction: The study addresses the need for an effective model to investigate *Vibrio* species and their interaction with hosts, focusing on disease dynamics in the oyster *Crassostrea gigas* in the North Sea. Specifically, the research explores the exposure of invasive sources to pathogens, notably *Vibrio kanaloae*. Emphasizing the importance of originality, the study utilizes molecular fluorescent in situ hybridization (FISH) techniques for rapid bacterial diversity identification in *Crassostrea gigas* from the island of Sylt, with 16SrRNA gene sequencing validating strain identification. Approach ensures a comprehensive understanding of the experimental design or approach, adhering to accepted scientific practices.

Purpose: The primary objective is to assess the dynamics of *Vibrio kanaloae* in *Crassostrea gigas* through FISH techniques, examining the response of different tissues to allopatric (T02) and sympatric (S12) strains. The purpose is to contribute valuable insights into the host-pathogen relationship, emphasizing the significance of using specific and controlled methodologies in studying Vibrio dynamics.

Methods: Employs molecular FISH techniques for rapid identification of bacterial diversity in *Crassostrea gigas*. Allopatric (T02) and sympatric (S12) *Vibrio kanaloae* strains are introduced to oysters, and various tissues (digestive glands, gill, and muscle) are examined using culture-independent methodologies. Rigorous controls, including negative controls, are implemented to ensure accurate and reliable results.

Results: The digestive glands, gill, and muscle in *Vibrio kanaloae* are identified as metabolically active through FISH techniques. Both strains (S12 and T02) are detected in the gill tissue, albeit with low colonization levels. Comparable numbers of *Vibrio* strains are observed in the digestive glands, while detection in muscular tissue is infrequent, indicating potential temporal distribution discrepancies.

Significance: The findings highlight the efficacy of FISH in rapidly assessing Vibrio dynamics in oysters, offering valuable insights into the interplay between *Vibrio* and the host-pathogen relationship. The study establishes a foundation for further investigations into the dynamics of *Vibrio* species in aquatic environments, contributing to the understanding of food protection and public health.

P1-152 Sea Cucumber against *Staphylococcus aureus*

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Introduction: *Staphylococcus aureus*-derived preformed enterotoxins pose a significant threat to global seafood safety, contributing to food poisoning incidents. This study aims to harness the medicinal potential of organic compounds present in marine organisms, specifically the sea cucumber *Holothuria leucospilota* from the Persian Gulf. The research delves into the antimicrobial properties of bioactive compounds within the whole bodies of these sea cucumbers.

Purpose: The primary objective is to explore and assess the antimicrobial capabilities of bioactive compounds extracted from *Holothuria leucospilota* against methicillin-resistant *Staphylococcus aureus* (MRSA) and Staphylococcal enterotoxin-producing strains (SEASA, SEBSA). The study seeks to contribute valuable insights into potential alternative treatments for bacterial infections associated with seafood consumption.

Methods: Bioactive compounds are extracted from *Holothuria leucospilota* using chloroform and methanol as solvents. The extracted compounds are then subjected to antimicrobial assays, employing disk diffusion tests, minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC) assays. Evaluation includes the assessment of inhibitory effects on the growth of MRSA and strains producing Staphylococcal enterotoxins.

Results: The study reveals that both methanol and chloroform extracts effectively inhibit the growth of all tested bacterial strains at MIC concentrations up to 100 mg/ml. Notably, the chloroform extract exhibits bactericidal activity against SEBSA at approximately 100 mg/ml, while concentrations below 100 mg/ml demonstrate bactericidal effects against MRSA and SEBSA. The methanol extract demonstrates the highest antibacterial activity among the extracts, showcasing their potential as effective antimicrobial agents.

Significance: Beyond their antimicrobial efficacy, the findings suggest a dual role for sea cucumber extracts as natural food preservatives, indicating a possible contribution to food conservation. However, the study emphasizes the need for further investigations to identify and separate active compounds within Holothuria leucospilota from the Persian Gulf. This research underscores the potential of marine organisms in providing solutions for seafood safety and public health, presenting a promising avenue for future studies in the field of antimicrobial research and food preservation.

P1-153 Evaluation of Various Sanitizers and Additives to Reduce *Listeria monocytogenes* on RTE Fishery Products

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Introduction: Ready-to-eat seafood such as smoked fish, sashimi, roe, and milt products are minimally processed and typically stored under refrigerated conditions where *L. monocytogenes* will survive and grow.

Purpose: Evaluate the efficacy of select sanitizers to reduce *L. monocytogenes* in minimally processed fishery products.

Methods: Cod milt, loose salmon roe and salmon sac roe were spray inoculated with a cocktail of *L. monocytogenes* to achieve a starting concentration of approximately 7-log CFU/g. The samples (100g) were placed into mesh bags and dipped in 100-200 ml of H_2O (control), sodium hypochlorite (Cl), peroxyacetic acid (PAA) or Prosur for various treatment times up to 60s then neutralized in buffered peptone water (BPW) containing sodium thiosulfate. Sample were stomached, further diluted in BPW and spread plated onto tryptic soy agar with 0.6% yeast extract (TSAYE) with a modified Oxford agar (MOX) overlay. Colonies were enumerated after incubation at 37°C for 48h.

Results: Cod milt treated with up to 10 ppm Cl or with up to 85 ppm PAA for 30s resulted in 0.12±0.08 and 0.21±0.18 log reduction of *L. monocytogenes* respectively. Loose salmon roe treated with up 150 ppm Cl or 150 ppm PAA for 60s and salmon sac roe treated with up to 200 ppm Cl or 200 ppm PAA for 30s did not result in any reduction of *L. monocytogenes*. All three products treated with 4000 ppm Prosur for up to 60s also did not result in any reduction of *L. monocytogenes*. In many instances, background microflora may have impact on *L. monocytogenes* enumeration since no pre-treatment of product was conducted.

Significance: Treatment of some seafood with sanitizers for 30-60s may not minimize the risk of *L. monocytogenes* contamination. Although sanitizers may require further optimization for efficacy and additional research is being conducted, the study also emphasizes the importance of following guidance and HACCP.

P1-154 Impact of Nisin on Proliferation of Pressure-Stressed and Wild-Type *Listeria monocytogenes* and *Listeria innocua* during a 5-Week Real-Time Shelf-Life Trial

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❖ ***Developing Scientist Entrant***

Introduction: With rapid implementation of high-pressure processing in various sectors of food industry, concerns associated with pressure-stressed microorganisms are emerging.
Purpose: As a GRAS-listed bacteriocin, nisin had been utilized in this study for controlling the proliferation of *Listeria monocytogenes* and *Listeria innocua* inoculated on smoked trout during a 5-week refrigerated shelf-life trial. Wild-type, and pressure-stressed phenotypes of the microorganisms were additionally compared in this study.
Methods: A 5-strain mixture of *L. monocytogenes* and one-strain *L. innocua* were used for target inoculation of 3.5 log CFU/g of smoked trout for a 5-week refrigerated (4.4 °C) real-time shelf-life trial. The pressure-stressed phenotypes were prepared by treating the surrogate and pathogen mixture at 15K PSI for 20 minutes (Hub880 barocycler, at 4.4 °C). Samples were (a) untreated control, (b) treated with sterile water, (c) treated with nisin. Results were statistically analyzed using ANOVA, followed by Tukey- and Dunnett's-adjusted mean separation at type I error level of 5%.
Results: *L. monocytogenes* multiplied extensively during the 5-week trial and counts were increased ($p < 0.05$) from 3.68 ± 0.1 log CFU/g on first week to 6.03 ± 0.1 log CFU/g. Both phenotypes and the surrogate microorganisms (*L. innocua*) illustrated similar ($p \geq 0.05$) multiplication trends. Unlike samples treated with water, nisin were effective ($p < 0.05$) to keep the microbial counts of week 5 lower compared with the controls. Counts of nisin-treated wild-type and pressure-stressed *L. monocytogenes* and *L. innocua* were 0.92, 0.53, 1.45, and 0.85 log CFU/g lower ($p < 0.05$) than the untreated controls, respectively. Similar trends were observed for background microflora of the product.
Significance: Our study illustrates that the selected surrogate microorganism has comparable sensitivity to nisin relative to *L. monocytogenes* and thus could be used interchangeably in future public health microbiological challenge studies with similar scope. Additionally, we observed that pressure-stressed *L. monocytogenes* has proliferation and sensitivity to nisin comparable to the wild-type pathogen.

P1-155 Assessing Customer Online Evaluation of Food Safety in Seafood Restaurants: Fresh, Visual, Smell, and Taste Perspectives

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❖ ***Developing Scientist Entrant***

Introduction: Seafood has been linked with several multistate foodborne illness outbreaks in the United States. Restaurant customers often relay their satisfaction or dissatisfaction with food through online reviews. Customers, in this study, identified freshness as the most frequent food quality aspect used to communicate food safety in restaurants in online reviews. Customers also used sensory cues, including smell, visuals, taste, and texture, to evaluate food.
Purpose*:* This study aimed to determine restaurant consumers' sensory cues to evaluate safety in seafood restaurants.
Methods: Customer reviews from New York City restaurants serving seafood were collected on Yelp. The dataset was cleaned to retain the reviews within a three-month period in 2022. Utilizing recommended qualitative analysis methods, reviews were analyzed line by line retaining only those reviews related to seafood safety. Codes were assigned to review segments and then grouped into themes. One hundred food safety reviews related to safety and freshness were manually analyzed from 213 seafood restaurants.
Results: Out of 100 reviews related to seafood freshness, reviews related to fresh taste, smell, and visual were 95, 2, and 11, respectively. Findings suggest that consumers relied on taste, visual cues, and smell to evaluate seafood freshness, providing customers with safety and quality assurance. Also, customers appeared to use visual cues and smell to form their pre-consumption evaluations, while taste appeared to affirm their post- consumption evaluations of seafood freshness.
Significance: This study shed light on seafood restaurant research indicating that taste was the most frequent sensory cue used by reviewers over this time period, followed by visual and smell. Through online reviews, customers expressed their evaluation of freshness and possibly formed their food safety risk perceptions.

P1-156 Investigating the Effects of Aquaculture Practices, Physiochemical Parameters and Extreme Weather Events on *Vibrio parahaemolyticus* in Oysters and Water from the Chesapeake and Delaware Inland Bays

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❖ ***Developing Scientist Entrant***

Introduction: *Vibrio parahaemolyticus* is an important human pathogen resulting in vibriosis via consumption of raw or undercooked oysters. Research shows rises in vibriosis rates thought to be attributed to climate change, reinforcing the need to understand the effects of extreme weather events (EWEs), physiochemical parameters (PPs), and aquaculture practices (APs) on *V. parahaemolyticus*.
Purpose*:* The objective of this study was to investigate the effects of PPs, APs and EWEs on the abundance of total and pathogenic *V. parahaemolyticus.*
Methods: Six oysters and 1L of water were collected from two different aquaculture practices (floating [FA] and bottom [BA]) from three different sites in the Chesapeake and Delaware Inland Bays biweekly from April-October 2022. Weather conditions and PPs were recorded during each collection. Oyster homogenates and water samples were tested for total (*tlh*$^+$) and pathogenic (*tdh*$^+$ and *trh*$^+$) levels of *V. parahaemolyticus* using MPN-qPCR.
Results: The detection rate of the *tlh* gene was 89.5% and 94.8% in the FA oysters and water, with log MPN/g/ml ranging from -0.824 to 4.311 and -2.824 to 1.631, respectively. In the BA oysters and water, the *tlh* detection rate was 92.3% and 97.4%, with log MPN/g/ml ranging from -0.824 to 3.484 and -2.824 to 2.631, respectively. The rate of detection for *tdh* and *trh* in FA were 60.5%, 64.1% and 55.3%, 64.1% and in BA were 59%, 61.5% and 61.5%, 71.8% for oyster and water samples respectively. The abundance of *tlh* in oysters, *tdh* in water, and *trh* in both oysters and water was higher in FA than BA. The differences between the practices were not significantly different ($p>0.05$). The abundance of *V. parahaemolyticus* varied in association with PPs, months, sites and EWEs.
Significance: This study provides a robust understanding of the effects of PPs, EWEs and APs on *V. parahaemolyticus* to make more informed decisions to improve public safety.

Poster

P1-157 Effects of High-Pressure Processing on the Organoleptic, Microbial and Chemical Qualities, and Bacterial Community of Escolar Meat during Cold Storage using High-Throughput Sequencing

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Introduction: High-pressure processing (HPP) is a non-thermal processing technology that can kill pathogenic and spoilage bacteria in food without affecting food quality characteristics, such as natural flavor and nutritional content, etc.

Purpose: This research aimed to determine the effects of HPP on microbe deactivation, delay of the loss of chemical and organoleptic qualities, and extension of storage life on the Escolar meat.

Methods: The HPP (300, 400, 500 and 600 MPa for 5 min) was applied to evaluate the microbial, chemical, and organoleptic properties of Escolar meat at 4 ℃ of storage. In addition, it was also determined that the differences in bacterial flora between untreated samples and samples subjected to 300 and 500 MPa by using high-throughput sequencing (HTS) technology.

Results: The results indicated that with an increase in pressure, the lightness (*L**), and whiteness (*W*), *ΔE* (color difference) and texture (hardness, cohesiveness, springiness and chewiness) increased, whereas redness (*a**), yellowness (*b**), aerobic plate count (APC), psychotropic bacteria count (PBC), H_2S-producing bacteria count (HBC), and coliform decreased. In addition, HPP at least of 300 MPa could delay the increase in microbial count and total volatile basic nitrogen (TVBN) value as well as the rising trend of pH. The results of organoleptic and TVBN showed that pressure treatment of 300-400 MPa and 500-600 MPa can extend the storage life from 3 days (untreated sample) to 6-12 days and 15 days, respectively. HTS revealed that HPP significantly changed the spoilage microbiota in samples after 15 days cold storage and delayed the spoilage process.

Significance: Overall, the results of this study confirmed that HPP treatment can effectively inhibit microbial growth, delay quality deterioration, and extend the shelf life of Escolar meat.

P1-158 Application of Novel Microwave-Assisted Induction Heating Technology for Extending the Shelf Life of Ready-to-Eat Rice through Microbial, Physical, and Chemical Quality Preservation

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Introduction: Microwave-assisted induction heating (MAIH) is a composite heating system that combines top-down microwave irradiation (1,000 W, 2,450 MHz) with bottom-up electromagnetic induction (1,500 W).

Purpose: This study aims to examine whether ready-to-eat rice prepared with MAIH outperforms traditional steaming in terms of delaying quality degradation during storage, thereby extending shelf life.

Methods: The rice cooked under optimal MAIH conditions (120℃ for 240 s and 100℃ for 300 s) as identified by our previous study, alongside rice prepared using conventional steaming methods (100℃ for 30 min), was stored at 35 ℃, 25 ℃, and 4 ℃. The study periodically assessed changes in microbial counts, as well as physical and chemical quality parameters.

Results: The results revealed that MAIH-treated rice demonstrated no detectable microbial growth at any storage temperature throughout the study. However, steamed rice exhibited microbial growth with increasing aerobic plate counts at 35℃, 25℃, and 4℃ after two, four, and sixteen weeks, respectively. Color analysis revealed a decline in *L** and *W* values, with increased *b** values over time for all samples at all storage temperatures. The pH values of the MAIH-treated rice remained stable, while steamed rice demonstrated a pH decrease during later stages of storage at 25℃ and 35℃. The hardness of MAIH-treated rice slowly increased at 25℃ and 35℃, whereas the steamed rice experienced a notable decrease. At 4℃, all samples demonstrated increased hardness, with steamed rice having significantly higher values than MAIH-treated rice.

Significance: Overall, MAIH treatment effectively delayed color, pH, and hardness alterations in rice in comparison to traditional steam cooking, demonstrating its potential for efficient pasteurization, reduced processing time, and improved overall quality of ready-to-eat rice.

P1-159 Culture Dependent vs. Culture Independent 16S Sequencing for Bacterial Communities during Spanish Mackerel Decomposition at 0□

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U.S. Food and Drug Administration, Dauphin Island, AL

Introduction: Bacteria produce chemical products in Spanish mackerel during decomposition at low temperature storage. Understanding temporal changes in bacterial community composition can potentially identify biomarkers for decomposition. Comparing culture independent and dependent sequencing results over time can illuminate both community shifts and practicality of sequencing methods to assess biomarkers of decomposition.

Purpose: The study objective was to evaluate bacterial community composition, comparing culture dependent and independent 16S sequencing methods during the storage of Spanish mackerel at 0℃ to determine if 16S sequencing methods could help identify biomarkers of decomposition.

Methods: Spanish mackerel were caught via hook and line in the Gulf of Mexico and immediately expired on ice. Whole gutted fish were incubated at 0℃ for 42 days. Triplicate samples were collected every seven days for three weeks, then every three days until day 42. A portion of each sample was processed and the 16S rRNA gene amplicon from primers 26F1 and 534R1 was used for metagenomic analysis (culture independent). The remaining portion of each sample was homogenized (1:10 in artificial seawater), spread plated on TSA, and incubated at 4℃ for 2 weeks. DNA from up to 48 colonies at each sampling point was purified and used for Sanger sequencing of 16S rRNA gene with 27F and 1492R primers (culture dependent).

Results: Culture dependent results showed *Pseudomonas* as the consistently dominant genus for the duration of the study. The culture independent method showed the initial bacterial community consisted primarily of the family Staphylococcaceae. By day 42, it consisted predominantly of the family Pseudomonadaceae, with a large shift in composition around day 21.

Significance: Evaluating different sequencing methods for assessing bacterial community compositions and their changes during decomposition may provide key insights into the most effective, rapid, and reliable methods to monitor biomarkers of seafood decomposition.

P1-160 Culture Dependent vs. Culture Independent 16S Sequencing for Bacterial Communities during Decomposition at 0□ in Red Snapper

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Introduction: Spoilage bacteria drive development of chemical compounds in seafood decomposition during low temperature storage. Understanding these bacterial communities and how their composition evolves during storage provides valuable insights into identifying biomarkers of decomposition. Culture independent and dependent sequencing yield valuable results for comparison in relation to understanding bacterial community composition.

Purpose: The study objective was to evaluate bacterial community composition using culture dependent and independent 16S sequencing methods during storage of red snapper at 0℃.

Methods: Red snapper were caught via hook and line in the Gulf of Mexico and immediately expired on ice. Whole gutted snapper were stored at 0℃ for 42 days. At each sampling point, weekly for weeks 1-3 and every 3rd day for weeks 3-6, triplicate samples were collected, and metagenomes analyzed using

the 16S rRNA gene amplicons with 26F1 and 534R1 primers (culture independent). Samples were homogenized (1:10 with artificial seawater), spread plated on TSA, and incubated at 4°C for 2 weeks. DNA from up to 48 colonies from each sampling point was purified for 16S rRNA gene sequencing via Sanger sequencing using 27F and 1492R primers (culture dependent).

Results: The culture dependent results showed the initial bacterial community consisted primarily of *Pseudomonas,* with few *Psychrobacter.* By day 42, it consisted almost exclusively of *Pseudomonas*. The culture independent method showed the initial bacterial community consisted primarily of *Staphylococcus*. By day 42, it consisted predominantly of *Pseudomonas*, with a large shift in the community around day 21.

Significance: Evaluating different sequencing methods for assessing these bacterial communities and their composition changes during temperature-controlled storage will provide key insights into the most effective, rapid, and reliable methods to monitor biomarkers of seafood decomposition.

P1-161 Detection of Histamine in Canned and Frozen Sardines and Anchovies Using a Rapid Lateral Flow Assay for Histamine Detection

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Introduction: As fish decompose, they can develop high levels of histamine. If ingested this can lead to scombroid poisoning in humans with symptoms such as rash, nausea, vomiting, and diarrhea. With an FDA action level of 50 ppm, it is important to have a reliable test kit for the detection of histamine within various species of fish.

Purpose: To evaluate the performance of a rapid lateral flow assay for histamine detection on canned and frozen sardines and anchovies.

Methods: Store bought canned anchovies, canned sardines, frozen anchovies, and frozen sardines were used for analysis. Using a qualified histamine spike stock samples were spiked at 10 ppm, 30 ppm, 100 ppm, and 200 ppm. All samples were extracted per the kit insert and high samples were diluted as necessary. Analysis was performed on two lateral flow histamine lots over two days with the %Recovery and %CV calculated.

Results: The acceptance criteria for each spike level were +/-20% recovery and a %CV of <20%. All spike levels for each sample met these criteria.

Significance: When released the analyzed rapid histamine lateral flow assay was only validated for tuna and fish meal. Validating the test kit on these additional commodities allows a broader range of customers to utilize the test kit to ensure more fish is safe to consume.

P1-162 Seafood Fraud: Is FSMA 204 & Traceability the Answer?

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Introduction: Seafood fraud is a pervasive issue in the U.S. seafood industry, with significant levels of mislabeling in both supermarkets and restaurants, prompting new regulatory measures like FSMA Rule 204 to enhance traceability and oversight.

Purpose: This abstract aims to highlight the extent of seafood fraud in the U.S., the new regulatory measures introduced by FSMA Rule 204, and the necessity for comprehensive industry cooperation to effectively combat this issue.

Method: THIS IS NOT A RESEARCH PAPER. THEREFORE, THERE WAS NO METHODOLOGY USED.

Results: The abstract concludes that FSMA Rule 204 enhances traceability efforts, but combating seafood fraud effectively requires collaboration with industry organizations and improved oversight.

Significance: The significance of this abstract lies in underscoring the critical need for comprehensive industry cooperation and standardized traceability to effectively mitigate seafood fraud beyond regulatory measures like FSMA Rule 204.

P1-163 Survey Results Toward Validating a Novel Low-Acid Seafood Recipe for Home Canning

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Introduction: Canning is widely recognized as a reliable method for preserving and maintaining the quality of foods. The National Center for Home Food Preservation (NCHFP) provides guidance on how to safely can a variety of produce, meats, and seafood. However, there is a limited number of NCHFP and USDA-validated guidelines for canning different types of seafood (n=7). The 2020-2025 USDA Dietary Guidelines recommends increasing seafood consumption given seafood's many health benefits.

Purpose: The purpose of this needs assessment was to survey home canners in the U.S. to prioritize future validated seafood recipes.

Methods: A literature review for unvalidated home-canning recipes for seafood was completed prior to developing a survey where participants were asked 28 questions, 5-10 minutes long, related to their views on novel canned seafood recipes, home-canning habits, and demographics. The survey was sent via snowball sampling through the Food Safety Extension Network. Those who self-identified from the US/Territories and 18 years or older could participate. Descriptive statistics were conducted.

Results: 502 complete survey responses were received. Data showed an emphasis on consumer interest in home canning of fish (34.13%), followed by peeled shrimp (18.96%), and seafood broth/stock (16.37%). Most of the surveyed home canners use a pressure canner (75.50%) and follow recipes through the NCHFP (47.11%) when canning fish. The majority of respondents identified as female (90.32%), rural environment (56.47%), and White (89.11%).

Significance: These survey results suggest many home canners are interested in canning seafood and support the development of additional validated seafood canning recommendations. A larger variety of validated seafood recipes on the NCHFP website will promote safe canning and also serve as a valuable resource for home canners who rely on these guidelines.

P1-164 The Emergence of Drug-Resistant Bacteria Isolated from Imported and Local Seafood Samples in the USA

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Introduction: Food imports are overlooked vehicles for introducing antibiotic resistance (ABR) determinants across country borders. Given that 65-85% of seafood products in the USA are imported, there is a notable risk of ABR dissemination into the US.

Objective: Here, we performed whole genome sequencing (WGS) on selected drug-resistant isolates retrieved from imported and local seafood samples in the USA in order to characterize their resistomes.

Methods: Between June and September 2022, imported (n=45) and local (n=18) seafood samples were aseptically collected from 8 retail grocery stores in Georgia, USA. Samples (~ 25 g) were homogenized in buffered peptone water (100 mL) and plated on the chromogenic RAPID' *E. coli*2 agar. Colonies were selected and their ABR profiles were assessed using the Kirby-Bauer disk diffusion assay against 19 clinically- and agriculturally important antibiotics. A subset of isolates (n=39) with unique ABR profiles were subjected to short-read WGS, and the genetic data were assessed using tools available at the Center for Genomic Epidemiology.

Results: Morphologically distinct isolates (n=39) were analyzed using WGS. The data showed that isolates belonged to various bacterial species, including *Providencia rustigianii* (n=11), *P. heimbachae* (n=3), *Serratia marcescens* (n=9), *S. grimesii* (n=1), *Aeromonas salmonicida* (n=13), *A.media* (n=1), and *Pseudomonas saponiphila* (n=1). The isolates exhibited high resistance to colistin MIC (4 to < 640 µg/mL) and were also resistant to other important antibiotics, including penicillin, streptomycin, tetracycline, meropenem, and erythromycin. WGS data also showed that the isolates harbored up to 7 acquired ABR genes (*tet(41)*, *tet(C)*, *aac(6')-Ic*; *aph(6)-Id*; *aph(3")-Ib*, bla_{SRT-2}; bla_{FOX}, and *sul(1)*). Notably, some isolates harbored *qacE,* which encoded resistance against antiseptic quaternary ammonium compounds. Additionally, the PathogenFinder software revealed that ten isolates were potential human pathogens.

Significance: This study highlighted the role of seafood as a reservoir for introducing and spreading ABR in the USA food supply chain.

P1-165 Evaluation of the Efficacy of Essential Oils (Cinnamon, Clove, and Thyme) Against Hepatitis A Virus Using Suspension and Carrier (Food-Contact Surfaces) Tests

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Introduction: Essential oils (EOs) have been used for centuries as flavor enhancers in foods, and due to their antimicrobial assets, they have potential as natural food preservatives.

Purpose: This study investigated the virucidal effects of three EOs –cinnamon, clove, and thyme – against hepatitis A virus (HAV) through suspension and carrier (food-contact surfaces) tests.

Methods: The initial titer of HAV was 1.8×10^7 log PFU/mL, and the inoculum was prepared using a 5% soil-load stock solution. HAV suspensions were mixed with each EO dose of 0.05, 0.1, 0.5, and 1% v/v for 1 h at room temperature (20°C) following ASTM E1052–11:2011 for suspension tests and also investigated in surface disinfection tests on hard food-contact surfaces (stainless steel and polypropylene) and soft food-contact surfaces (low-density polyethylene and kraft paper) according to the OECD:2013. After treatment, RT-qPCR was used to examine changes in HAV genome copies in suspension and carrier tests. Each treatment was conducted in three replications.

Results: In the suspension tests, EOs showed a dose-dependent impact; at the maximum dose of 1%, HAV titers decreased by ~1.60 log PFU/mL ($p < 0.05$). Surface disinfection tests also demonstrated the antiviral effect of EOs. In surface disinfection tests, EOs treated at 1% reduced ~2 log PFU/mL on stainless steel and polypropylene and ~2 and 1.4 log PFU/mL on low-density polyethylene and kraft paper ($p<0.05$), respectively. Notably, the carrier tests proved more accurate in assessing the true disinfection potential of EOs on food-contact surfaces, highlighting their practical applicability. Also, RT-qPCR results showed that HAV genome copies were not significantly ($p>0.05$) decreased until treated with a high dose (1%) in suspension and carrier tests.

Significance: These findings advance our knowledge of EOs as antimicrobials and also shed light on the potential of these EOs to serve as active surface disinfectants to reduce viral contamination, contributing to enhanced food safety practices in the industry.

P1-166 Ultralow Temperature Enhances HPP Inactivation of Viruses

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Introduction: Noroviruses and caliciviruses are moderately sensitive to high pressure processing (HPP) above 0°C with >400 Megapascals (MPa) or higher required to inactivate virus.

Purpose: Colder temperatures enhance inactivation, ultracold temps at c.a. -35°C were tested to determine if even greater inactivation is observed.

Method*:* HPP sensitivity of murine norovirus (MNV) and Tulane virus (TV) ultracold and to ice phase transitions were evaluated. Identical samples containing MNV or TV were either equilibrated to +1.5°C (thawed) or -40°C (frozen) 24 h prior to pressurization. All samples (thawed and frozen) were then placed in a pre-chilled pressure chamber which was then rapidly filled with -40°C chamber fluid. Virus samples were immediately pressurized for 5 min at 200, 250 or 300 MPa. Controls (untreated) were not pressurized. Statistical significance was assigned using a student t-test when P values of < 0.05 were observed. (n=3; n=9)

Results: For samples that were thawed and then pressurized in -40 C chamber fluid, the MNV average log reductions at 200 MPa was 4.4, while >6.1 log reduction (non-detectable) was achieved at 250 and 300 MPa. TV samples averaged 2.3, 5 and 4.3 log reduction at 200, 250, and 300 MPa respectively. For samples that were frozen and then pressurized in -40 C chamber fluid, the MNV average log reductions were 2.3, 3.2 and 4.2 at 200 MPa, 250 MPa and 300

MPa, respectively, while TV samples averaged 0.81, 2.3 and 1.7 log reductions at 200, 250, and 300 MPa, respectively. Inactivation of TV within oysters at these pressures was also demonstrated.

Significance: Results demonstrate enhanced inactivation of norovirus surrogates compared to refrigeration temperatures, and ultra-cold HPP performed on thawed samples especially enhances inactivation. This suggests that frozen foods could be treated by HPP and that there may be substantial advantage to freezing and pressure treating foods simultaneously.

P1-167 Optimizing and Comparing Viral Recovery from Polydimethylsiloxane (PDMS) Topomimetic Artificial Leaf Surfaces and Fresh Leafy Green Surfaces

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Introduction: Polydimethylsiloxane (PDMS) replicasts (topomimetic artificial leaf surfaces) have been used to elucidate the role of the produce surface waterscape on foodborne virus adhesion and removal, yet it is unclear if replicasts can be used as a model system for fresh leafy green surfaces without additional modifications.

Purpose: Validate that PDMS replicasts with hydrophobicity modifications can be substituted for fresh leafy green surfaces for complete virus recovery.

Methods: PDMS replicasts of 15- and 45-day old leaves of romaine lettuce plants were generated with flat PDMS replicasts (no surface topography) included as controls. The average contact angle of four 10 µl sessile drops of phosphate buffered saline (PBS), with and without a surfactant to increase surface wettability, was determined with an optical tensiometer for replicasts and fresh leaves. Coupons of replicasts and fresh leaves were inoculated with 10^5 PFU of Tulane virus (TV) supplemented with 0.05% Tween20 in triplicate. Inoculated coupons were agitated in ultrapure water before transferring to a rinse solution containing 0.05% Tween20 in PBS. TV titer was determined by plaque assay in both solutions.

Results: The average contact angle on flat PDMS replicasts was found to be 107.5°± 2.7 with pure PBS and 74.4°± 2.2 with PBS supplemented with Tween20. The average contact angle of PBS on fresh romaine lettuce was found to range from 73.7° to 82.1°, depending on leaf age and axis. A one-step washing procedure resulted in a 1-1.4 log PFU/mL loss in TV recovery from fresh samples and complete recovery from replicasts. In a two-step washing procedure, less than a log difference in virus recovery was found between fresh and replicast samples without significant differences between leaf age or axis.

Significance: PDMS replicasts with surface modifications demonstrate similar virus recovery to fresh leaves, indicating utility of this model system in future experimentation.

P1-168 Exploring the Potential of Deep Eutectic Solvents in Concentrating Non-Enveloped Virus for Improving Rapid Virus Detection

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✦ ***Undergraduate Student Award Entrant***

Introduction: Concentration of noroviruses from foods is a crucial step for effective viral detection. Deep Eutectic Solvents (DESs) are an emerging class of reagents that are composed of Lewis or Bronsted acids and bases that form eutectic mixtures that have adjustable properties, including charge, depending on the ratios of the components in the solvent. Both synthesis and purification of DESs is environmentally friendly and cost efficient. Although promising, the potential of DESs to serve as a reagent for concentration of noroviruses prior to detection has not been evaluated.

Purpose: The purpose of this study was to examine the ability of DESs to concentrate bacteriophage MS2, a human norovirus surrogate.

Methods: MS2 was diluted to 10^5 PFU/mL in PBS, vortexed with different DESs (cobalt, nickel, and dysprosium-based) and distilled water (control). Separation of DES-bound targets was completed using a magnet. The supernatant was removed and samples were washed to remove the unbound virus. Captured MS2 was eluted by vortexing with modified Luria Broth, and recovered virus quantified by RT-qPCR.

Results: The cobalt-based DES displayed 65.8% capture and 32.9% recovery; the nickel-based DES displayed 59.2% capture and 36.3% recovery; and the dysprosium-based DES displayed 98.5% capture but only 2.12% recovery (n=3). Thus, the DESs displayed promising capture results, though precent recovery of purified RNA was notably low for all DESs compared to the initial titer, especially dysprosium. This suggests that further optimization of elution conditions could improve recovery.

Significance: These results indicate that DESs have the potential to recover significant amounts of non-enveloped viruses and have potential to serve as a green, cost-effective reagent for concentration of viruses from food and environmental samples.

P1-169 Genomic Diversity and a Novel Phylogenomic Classification of Genus Norovirus

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Introduction: Noroviruses (NoVs) are traditionally classified based on the sequences of VP1 (genotype), RdRP (P-type), or dual-typing nomenclature. However, VP1 displays high genetic diversity, whereas RdRP exhibits low genetic diversity. Thus, current classifications solely relying on specific proteins may be insufficient and potentially lead to misleading results.

Purpose: We designed two panels specifically for sequencing the entire genome of NoVs. We performed an extensive genomic analysis using 1417 genomes obtained from NCBI.

Methods: We utilized our newly developed Next-Generation Sequencing (NGS) panel to sequence NoVs and assemble the genomes of ten samples. We conducted a comprehensive analysis including pairwise distance matrices, phylogenetic trees and sequence similarity networks to assess their genomic diversity and evolutionary relationships.

Results: Phylogenetic analyses based on the maximum likelihood (ML) and Bayesian inference methods and pairwise distance matrices of genotypes and P-types revealed unclear taxonomic distinctions. In particular, intra-genogroup identities of VP1 were 67–75% in GI, 65–87% in GII, 73% in GIII, 68–74% in GIV, 68% in GV, and 65% in GVI. Among the alignments, identity scores of 80% or higher were only evident in genotypes GII.22–GII.27, GII.NA1, and GII.NA2, which were identified recently. While inter-genogroup identities were mostly below 60%, certain genotypes within GIV, GVII, GVIII, and GIX manifested genome similarities exceeding 60% when compared to some or all of the genotypes in other genogroups. Our phylogenomic approaches and sequence similarity networks demonstrated that genogroups GVIII and GIX are nested within GII and certain strains of GIV and GVI form host-based clusters, suggesting potential zoonotic transmission. We introduced a novel classification system, "D-types" relying on the entire genome sequences. This classification can distinguish each dual type by genomic characteristics, even identifying intragenotype recombinant strains of GII.4.

Significance: Our study represents a crucial initial step towards the phylogenomic classification of genus *Norovirus*. This is valuable for not only interpreting the evolutionary relationships among NoV strains but also developing antiviral targeting strategies.

P1-170 The Persistence of Murine and Human Norovirus Through a Simulated Gastrointestinal Tract

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❖ ***Developing Scientist Entrant***

Introduction: The human norovirus (HuNoV) is the main cause of gastroenteritis worldwide, however, the behavior of the HuNoV throughout the human gastrointestinal tract poorly is unknown.
Purpose: The aim of this study was to evaluate the persistence of the murine norovirus (MNV-1) and the HuNoV through simulated gastrointestinal digestions of at-risk food matrices.
Methods*:* The persistence of the MNV-1 (6 log PFU/mL) and the HuNoV (8 log genome copies/mL) was studied using the virus alone in water and on experimentally contaminated food, such as raspberries, strawberries, lettuce, and oysters. Two recognized digestive models were used in this study: the INFOGEST protocol (static digestion), in triplicta and the TIM-1 (dynamic digestion), in duplicata. The persistence of MNV-1 and HuNoV was studied at different steps: after chewing in the mouth, after incubation in the stomach and the small intestine. In dynamic digestion, samples were taken in the stomach, the duodenum, the jejunum, and the ileum at different times. To quantify the virus, plaque assays and RT-qPCR combined with a PMAxx treatment were used.
Results: After static digestion with MNV-1, there was a significative difference at the end of the digestion compared to the initial virus (6.02 ± 0.04 log) (one-way ANOVA, Tukey, $p<0.05$) for raspberries (5.19 ± 0.11, strawberries (5.22 ± 0.39 log), and oysters (4.68 ± 0.56 log). After the gastrointestinal digestion with HuNoV, there was no significative difference (one-way ANOVA, Tukey, $p>0.05$) as the initial virus was 8.19 ± 0.57 log genome copies/mL, and the viral recovery were 7.98 ± 0.67 log 7.50 ± 0.52 log and 8.15 ± 0.60 log for the virus alone, the raspberries and the oysters, respectively. Results obtained with the TIM-1 have shown a similar trend.
Significance: The data generated during this study confirmed that viruses persist following static and dynamic digestion, offering a better understanding of the pathogenesis and its impact on infective dose.

P1-171 Deposition of Viral Aerosols on Surfaces and Food

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Introduction: The human norovirus (HuNoV) is the main cause of viral foodborne diseases. The manifestation of the disease through symptoms such as diarrhea and vomiting allow for the release of the virus into the environment.
Purpose: Since little is known about this route of transmission of foodborne viruses, there is a need to determine the role of air (aerosols) in the contamination of food and food preparation surfaces.
Methods: Ten (10) soda lime glass, 10 polypropylene plastic and 10 stainless-steel discs were deposited on a glass plate introduced into the aerosolization chamber. Phage PhiX-174 lysate was used as model at a concentration of 109 PFU/mL and was introduced into the nebulizer. After a 10-minute nebulization, a 2-hour rest period was observed. The air in the chamber was sampled, and each type of discs collected were suspended separately in 5 mL of phage buffer. The amount of phage collected in the air and on the discs was determined by the double-layer method. Tests were carried out in triplicate.
w At a relative humidity between 60 and 80%, the infectious phage was detected both in the air and on all three types of discs after the rest period at a concentration of 103 and 104 PFU/mL respectively. No significant($p>0.05$, one way ANOVA) differences were observed on infectious phage levels on the glass, stainless steel, and plastic discs. More experiments will be performed with other NoV models.
Significance: This study can bring information on the behavior of viruses in the air and after aerosolization. That knowledge can be used to better adapt control strategies in the environment.

P1-172 Survival of Norovirus Surrogate Bacteriophage MS2 in Arugula (*Eruca sativa*) and Beet (*Beta vulgaris*) Microgreens during Storage

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Introduction: Norovirus (NoV) outbreaks have been widely associated with fresh produce. Microgreens are young seedlings of vegetables and herbs often used as a garnish to add unique flavors and colors to a dish. Arugula and beet are among the most popular microgreens because of their spicy flavor and vibrant color, respectively. Little is known about the survival of viruses in these microgreens during refrigerated storage.
Purpose: This research assesses the survival of NoV-surrogate bacteriophage MS2 in arugula and beet microgreens during refrigerated storage.
Methods: Five gram samples of 7-day old arugula and beet microgreens were spot inoculated with MS2 (8 log PFU/g), placed in polypropylene boxes with lids and stored at 5°C. MS2 survival was evaluated every 24 h for ten days. Propagation of MS2 was done using it's *Escherichia coli* C3000 host on tryptic soy agar (TSA) plates using the double agar overlay method. TSA plates were incubated overnight at 37°C, and viral titer was determined. Experiments were performed in triplicate using three biological replicates. Data were analyzed using ANOVA and Student *t* test ($p<0.05$).
Results: MS2 concentration did not decrease significantly over the first five days of refrigerated storage in arugula or beet microgreens. After eight days of storage MS2 concentration decreased significantly by ~1.5 Log PFU/g in arugula. A similar significant decrease in MS2 was observed in beet microgreens after 10 days of storage.
Significance: Results show good survival of MS2 in microgreens during storage and suggest the same for NoV. MS2 stability in microgreens indicate that NoV contaminated microgreens may pose a risk when added to foods as a garnish. Findings also suggest virus survival may differ for different microgreens.

P1-173 Unveiling the Correlation Between Infectivity Assay and RT-qPCR in Norovirus Detection Using Tulane Virus as a Surrogate

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❖ ***Developing Scientist Entrant***

Introduction: Human norovirus is globally acknowledged as the primary cause of viral foodborne illness. The absence of a robust cultivation system requires employing RT-qPCR for downstream detection and quantification. However, the efficacy of RT-qPCR in distinguishing infectious from non-infectious particles compared to infectivity assays remains unclear.
Purpose: To explore the correlation between the infectivity (plaque) assay and RT-qPCR for the quantification of norovirus, using Tulane virus as a cultivable surrogate.
Methods: Tulane virus lysate was purified through gradient ultracentrifugation and adjusted to a working concentration of 7 log10 plaque-forming unit (PFU)/mL in Tris-EDTA buffer (pH=7.2). Serially diluted virus stock underwent RNA extraction, with or without RNase pre-treatment, and was then quantified with a probe-based RT-qPCR. The conventional RNase pre-treatment degrades free RNA in the virus suspension, thereby enhancing the detection of intact, presumptively infectious virus particles. All treatments have been done in triplicate.

Results: A strong correlation was found between log10 genome copies (GC) and log10 PFU per reaction for both RNase-treated and untreated samples, with a Pearson's product moment correlation coefficient of 0.99. The RNase pre-treated samples showed a 0.2±0.1 lower log10 GC per reaction across the dilutions (p<0.05), signifying the integrity of the purified virus stock. In comparison to the infectivity assay, RT-qPCR quantified Tulane virus particles at 3.4±0.1 and 3.7±0.1 log10 higher when samples were treated with and without RNase, respectively (t-test, p<0.05). This underscores the sensitivity of RT-qPCR and its potential to overestimate infectious virus particles. However, the latter assumption does not account for the actual multiplicity of infection when performing a plaque assay.

Significance: The outcomes of this study will contribute to the more accurate estimation of infectious norovirus particles in food and environmental samples through the establishment of the correlation between the virus quantification data obtained from RT-qPCR and infectivity assay.

P1-174 A Novel Method for Capturing and Concentrating Human Norovirus Using Engineered *E. coli*

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Introduction: Human noroviruses are the leading cause of non-bacterial gastroenteritis globally. A significant challenge in managing human noroviruses is the absence of an effective method to concentrate viruses from food and environmental samples before detection. Current norovirus concentration techniques face various limitations, including high cost, scalability issues, and generally low-capture efficiency (<30%). Therefore, we need a new human norovirus concentration method to overcome these hurdles.

Purpose: This study aimed to develop a novel norovirus concentration method by engineering nonpathogenic *Escherichia coli* (*E. coli*) to express norovirus-affinity peptides.

Methods: Human norovirus affinity peptides were cloned at the C-terminus of ice nucleation protein (INP) and expressed in *E. coli* BL21. The engineered *E. coli*'s ability to capture Norovirus GII.4 Sydney was assessed through a suspension assay using RT-qPCR, calculating the reduction of the input virus in the suspension. Statistical comparisons were performed by one-way ANOVA. A P-value of <0.05 was considered significant. Additionally, the interaction between the expressed peptides on *E. coli* and VLP (a substitute for infectious GII.4) was visualized through TEM imaging.

Results: Capture efficiencies varied between 66% (SD±4.0) and 81% (SD±0.24) across 7 engineered *E. coli* strains with different peptides, and *E. cloacae*, a bacterium naturally binding to norovirus. The highest capture efficiencies observed with the engineered *E. coli* strains, 81.34% (SD±0.2) and 76.55% (SD±1.0) were higher than that observed with *E. cloacae* (76%, SD ±3.0), as well as the no-peptide scaffold control. The visualization of norovirus particles attached to the engineered *E. coli* was achieved through TEM, consistent with similar capture efficiencies observed in the pull-down assay.

Significance: This is the first capture and concentration method targeting human norovirus using engineered bacteria. Due to its simplicity in development, cost-effectiveness, and scalability for processing larger volumes, we anticipate that this approach can be readily adopted for concentrating norovirus from food, patients, and environmental samples.

P1-175 Inactivation of Rotavirus Using Chemical Treatment on Fresh Vegetable

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Introduction: Rotavirus infections are a leading cause of severe, dehydrating gastroenteritis in children <5 years of age despite the global introduction of vaccinations for rotavirus. They are frequently linked to fresh vegetables, leading to foodborne illnesses. Therefore, effective control strategy should be required to reduce the transmission of this virus on fresh vegetables.

Purpose: This study investigated the inhibition effect of chlorine dioxide (ClO_2) and peracetic acid (PAA) in virus suspension, on a food contact surface (stainless steel), and on the food (lettuce) contaminated with rotavirus.

Methods: The suspension test used 100 µl of virus in 900 µl of disinfectant. The surface test used 50 µl of virus-laden media on 1 cm of stainless steel with 100 µl of disinfectant. The food test, lettuce was cut into 2 cm, inoculated with 100 ul of virus, and treated with 10 ml of disinfectant. All experiments were performed in triplicate, with processing times of 1 min and 5 min. Treatment of ClO_2 and PAA (0-20, 0-120 ppm for suspension; 0-300, 0-2500 ppm for food contact surface; 0-50, 0-200 ppm for food) were individually conducted on each sample. Viral titers were measured by plaque assay, and RV reduction was analyzed using one-way ANOVA and Duncan's multiple-range test, with significance set at $P < 0.05$.

Results: The virus was completely inactivated at 20 ppm of ClO_2 and 120 ppm of PAA respectively in the suspension, and the virus was not detected at 200 ppm of ClO_2 and 2500 ppm of PAA in the stainless-steel samples (4 log PFU/mL reduction for both disinfectants). The highest reduction was achieved (1.79, 2.25 log PFU/mL reductions) at 50 ppm of ClO_2 and 200 ppm of PAA on lettuce respectively. Viral reduction values significantly increased (p<0.05), as increasing disinfectant concentrations.

Significance: The study showed ClO effective at low concentrations, indicating its potential for preventing RV contamination in the fresh vegetable industry.

P1-176 Impact of Hypoxia on Human Norovirus (HuNoV) Replication in 2D and 3D Culture Models

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Introduction: Both 2D cell culture and 3D organoid culture methods have emerged as valuable tools for mimicking the human system in virus infection experiments.

Purpose: To investigate the relationship between human norovirus (HuNoV) proliferation and hypoxic conditions, we examined HuNoV replication in 2D induced pluripotent stem cells (iPS) and 3D human intestinal organoids (HIOs).

Methods: 2D iPS and 3D HIOs were cultured in 48-well plates. For 2D iPS, cells were treated with 10 or 50 µM $CoCl_2$ for 24 hours, induced into a hypoxic environment, and inoculated with HuNoV. Supernatant was collected after 1 hour, and qPCR was used to analyze virus replication, tight junctions, hypoxia-related genes, differentiation of intestinal epithelial cells, and immune system and virus infection-related gene expression. For 3D HIOs, cells were treated with 200 µM bile acid and 50 µM $CoCl_2$ for 48 and 24 hours, respectively, followed by HuNoV inoculation and 4 days of cultivation for qPCR-based replication measurement.

Results: Following HuNoV inoculation, a 3.17 log increase was observed after 1 hour in the unbinding supernatant treated with 50 µM $CoCl_2$. Over a week of cultivation, HuNoV in cells and supernatant increased by 3.38 log compared to the initial inoculum, indicating rapid replication within an hour. 2D iPS cells exhibited high infectivity. Co-treatment with 50 µM $CoCl_2$ and HuNoV infection led to increased expression of ZO-1, VEGF, Villin, Muc-2, IFN-β, TRL3, and ISG56 genes, while HIF-1α expression decreased. In 3D human organoids, bile acid (200 µM), $CoCl_2$ (50 µM), and combination treatment increased replication by 24, 52, and 41 times, respectively.

Significance: The study sheds light on HuNoV replication in hypoxic conditions, offering insights into food protection and public health. The findings emphasize the importance of considering the impact of environmental factors on virus infectivity in food safety protocols.

P1-177 Ultraviolet Light Systems for the Inactivation of Hepatitis A Virus on Food Contact Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Ultraviolet-light (254-nm UV-C and 279-nm UV-C LED) technologies are being researched for surface decontamination and antiviral effects. Hepatitis A virus (HAV) outbreaks continue to occur in food environments via food contact surfaces.

Purpose: This research aimed to determine the inactivation of HAV on stainless-steel (SS) and ceramic surfaces (CS) using 254-nm and 279-nm UV-C systems.

Methods: One hundred µl of HAV at ~5.5 log PFU/mL was aseptically spread on 6 sterile 3x3 cm^2 SS or CS coupons within sterile petri-dishes, air-dried, and treated with either 254-nm UV-C or 279-nm UV-C LED for 0 to 3.75 min. Control and treated viruses were recovered using ten-fold serial dilutions in Dulbecco's Modified Eagle's Media containing 2% fetal bovine serum. Plaque assays using confluent FRhK-4 for HAV were performed within 6-well plates. Data from three replicate trials were statistically analyzed using mixed model analysis of variance with Tukey's adjustment ($p \leq 0.05$). The linear model was used to calculate the dose (D-value) required to decrease the infectivity of HAV by one-log.

Results: HAV on SS coupons treated with 254-nm UV-C (doses of 0 to 54 mJ/cm^2) showed 2.99±0.29 log reduction after 1.5 min and 3.63±0.09 log reduction after 2.5 min, while reductions with 279-nm UV-C LED (0 to 19.05 mJ/cm^2) were 1.96±0.02 log PFU after 1.5 min and 2.75±0.15 log PFU after 2.5 min. HAV on CS after treatment with 254-nm UV-C (doses of 0 to 60.8 mJ/cm^2) showed 2.32±0.11 log reduction after 1.5 min and 3.35±0.09 log after 2.5 min, while reductions with 279-nm UV-C LED (0 to 28.6 mJ/cm^2) were 1.97±0.12 log and 3.39±0.10 log, respectively. D_{10}-values for HAV on SS and CS with 254-nm UV-C were 12.7 mJ/cm^2 and 16.9 mJ/cm^2, respectively, while with UV-C LED were 6.1 mJ/cm^2 and 7.7 mJ/cm^2, respectively.

Significance: These UV-C inactivation parameters can be used to design optimized UV-C technologies for HAV inactivation in food environments.

P1-178 Cultivation of Wild-Type Hepatitis A Virus Using Induced Pluripotent Stem Cell-Derived Hepatocytes

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❖ ***Developing Scientist Entrant***

Introduction: Hepatitis A virus (HAV) is a foodborne virus causing acute hepatitis in humans. Since the first HAV mandatory surveillance system was implemented in 2008, the number of hepatitis A patients in Korea in 2019 was recorded at 17,638, the highest ever. Although the HAV HM-175 strain can be cultivable in FRhK-4 cells in vitro, cultivating wild-type HAV (wtHAV) is still challenging.

Purpose: This study develops a novel wtHAV cultivation method using induced pluripotent stem cell (iPS)-derived hepatocytes.

Methods: wtHAV genotype IA serum from a hepatitis A patient was used for inoculum. For the cell culture model, a human iPS cell line and hepatocyte differentiation kit were used. Several iPS cell lines were differentiated into 4 lineages: hepatocyte progenitor stem cell (HpSC), hepatoblast (HB), immature hepatocyte (immHep), and derived mature hepatocyte (dHep). wtHAV was inoculated to each lineage of differentiated cells and cultivated. All HAV genome copies were quantified with one-step RT-qPCR.

Results: All 4.0 log viral genome copies/mL of inoculum were recovered after 1 hour of incubation. The wtHAV genome copy number increased after 3 dpi (LOD: 2.5 log/mL). The wtHAV viral genome copies in each hepatocyte lineage reached 6.16±0.13 log/mL in HpSC, 5.47±0.09 log/mL in HB, 4.93±0.18 log/mL in immHep, and 4.19±0.14 log/mL in dHep. The other iPS cell line also showed 6.48±0.03 log/mL, 6.29±0.00 log/mL, and 6.25±0.33 log/mL in HpSC, HB, and immHep, respectively. However, dHep was not effective in cultivating wtHAV *in vitro*.

Significance: This study developed a reliable technique to cultivate wtHAV using iPS-derived hepatocytes.

P1-179 Hepatitis A Virus Genome Recovered by Using Tiling Multiplex Amplicons and Oxford Nanopore Sequencing

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Introduction: Hepatitis A virus (HAV) is the most common cause of acute viral hepatitis in humans and is considered one of the leading causes of foodborne viral illness. HAV is primarily transmitted through common source outbreaks (e.g. food and water-borne) or through person-to-person contact via the fecal-oral route. Development of rapid and suitable whole viral genome sequencing methods for HAV is critical for better understanding virus origin as well as spread and will also facilitate source identification during outbreak investigations.

Purpose: To develop a rapid and cost-efficient multiplex PCR amplicon-based whole genome sequencing method to characterize HAV genome.

Methods: Novel multiplex PCR primer panels were designed to generate overlapping amplicons (~400bp) to cover approximately 7.5-kb of the HAV genome. The amplicons were sequenced on Nanopore GridION and Illumina MiSeq. The sequence data were trimmed to remove low-quality reads and adapter sequences for further analysis. Viral consensus sequences were extracted at ≥10x read depth using a reference alignment-based approach.

Results: Near complete genome coverage of HAV was achieved using the tiling multiplex PCR amplicon-based Nanopore sequencing method. A consensus sequence with 96.8% genome coverage and 214x average read depth was obtained with as few as 4,000 Nanopore reads starting with RNA sample in ~2 days. This viral consensus sequence displayed a sequence identity of 99.99% to the reference genome of HAV strain HM175/18f. The Illumina platform generated a consensus sequence with 98.3% genome coverage and 73969x average read depth from the same virus sample with a nucleotide sequence identity of 99.99% compared to that of HM175/18f using the same amplicon panel. A sequence similarity of 99.97% was observed between the Illumina and Nanopore-generated consensus sequences from the same virus strain.

Significance: This study demonstrated that the tiling multiplex PCR amplicon-based method could generate high coverage throughout the HAV genome and worked well on the Nanopore and Illumina platforms tested.

P1-180 Comparison of Pretreatments to Distinguish Infectious and Non-Infectious Foodborne Viruses

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne viruses, such as hepatitis A virus (HAV) and human norovirus are responsible for multiple foodborne illnesses. Molecular methods, such as RT-qPCR, are used as detection method, but it cannot discriminate between infectious and non-infectious viruses. Specific treatments prior to detection could overcome this problem by only detecting RNA from infectious virus in food samples.

Purpose: The aim of this project was to analyze the effectiveness of different RT-qPCR pretreatments applied to inactivated HAV.

Methods: HAV in suspension (1 x 10^5 PFU/mL) was totally and partly inactivated by heat (100 °C for 10 min or 80 °C for 1 min), pulsed light (7.20 J/cm^2 or 1.44 J/cm^2) and sodium hypochlorite (NaOCl, 500 ppm for 5 min or 200 ppm for 1 min), respectively. Inactivated HAV was treated with five different pretreatments: 50 uM PMA or PMAxx in 0.5% Triton X-100, 500 uM $PtCl_4$, magnetic silica beads and Amicon® centrifugal 100 kDa filter. Viral RNA was then extracted and amplified by RT-qPCR. All experiments were performed in triplicate.

Results: Results showed that PMA and PMAxx were significantly (2-way ANOVA followed by Turkey test, $p < 0.05$) the most effective treatments, on the

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total heat inactivation samples (2.61 ± 0.44 log reduction and 5.90 ± 0.35 log reduction, respectively). After heat inactivation, silica beads and centrifugal units were not as effective to remove free HAV RNA with a reduction of 0.75 log and 0.57 log, respectively. $PtCl_4$was the least effective pretreatment (0.13 log reduction) after the heat inactivation. All tested pretreatments combined with pulsed light and sodium hypochlorite inactivation methods failed to remove free HAV RNA (<1 log).

Significance: Implantation of pretreatments in standardized methods prior to RT-qPCR could reduce food recalls caused by the detection of viral RNA from non-infectious viruses.

P1-181 Matrix Extension of the Concentration, Extraction, and Detection of Enteric Viruses in Finfish

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Introduction: Consumption of contaminated seafood is a common source of gastroenteritis due to norovirus and hepatitis A virus (HAV). Shellfish is a commodity commonly associated with enteric virus outbreaks; however, outbreaks occur in other seafood matrices. In 2016, over 200 individuals became ill with HAV where scallops were implicated. In 2017, HAV was detected in imported tuna. These incidents demonstrated a need for methods for seafood commodities, outside of established matrices, that are not typically associated with viral outbreaks.

Purpose: This study expands the tuna matrix in the FDA's Bacteriological Analytical Manual (BAM) Chapter 26 to additional finfish, including a high fat fish (e.g., salmon) following FDA matrix extension guidelines.

Methods: Following FDA's BAM Chapter 26, 50g inoculated salmon (n=30) were processed using murine norovirus (MNV) as an extraction control. Norovirus and HAV were concentrated using ultracentrifugation, then RNA extracted with silica column-based kits. This study included samples with high (n=5), medium (n=15), and low/ limit of detection (LOD) (n=5) inoculation levels, with each tested in triplicate by RT-qPCR. Norovirus GI and GII levels were 670, 67, 6.7 genomic copies/g and HAV levels were 10, 1, 0.1 plaque forming units/g, respectively.

Results: Little to no inhibition was observed in all replicates for the detection of MNV, norovirus GI and GII, and HAV. MNV extraction control was detected in all samples and results were acceptable. Detection frequencies in the samples with high, medium, low/LOD inoculum levels were 100%, 93%, and 60% for norovirus GI; 100%, 84%, and 7% for norovirus GII; and 100%, 82%, and 33% for HAV, respectively.

Significance: This salmon matrix extension, along with an ongoing amberjack matrix extension, will complete the requirements for a "finfish" category in the FDA's BAM. Validated methods for extraction of enteric viruses from additional seafood matrices will help provide regulatory laboratories the tools necessary to respond to future foodborne viral outbreaks and conduct surveillance sampling, as needed.

P1-182 Cell Line Infectivity Assay May Not be Suitable for Validation of High Pressure Processing against *Cryptosporidium parvum* in Apple Juice

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❖ ***Developing Scientist Entrant***

Introduction: Current validation studies determine reductions of *Cryptosporidium parvum* using infectivity in cell line models; however, infectivity assays are costly and may not be suitable for assessing *C. parvum* inactivation in high pressure processing (HPP) validation studies.

Purpose: To evaluate the suitability of cell line infectivity assay for determining *C. parvum* inactivation in apple juice using HPP.

Methods: Apple juice was inoculated with *C. parvum* and treated by HPP at 400-600 MPa for 3 min. Treated and untreated control oocysts were recovered by centrifugation, washed and transinfected into human colon cancer cell line, HCT-8, where infectious oocysts attached to cells in the initial 1-hr infection and non-infectious oocysts were washed away. Subsequent infection occurred at 37°C, 5% CO_2 for an additional 48 hr. Microbial reductions were determined using real-time PCR (qPCR) targeting the *hsp70* amplicon. The study was repeated three times independently and each repeat consisted of two technical duplicates at each step.

Results: Standard curve fitness of qPCR following cell line infection with serial diluted oocysts (R^2=0.89-0.92) was lower than serial diluted oocysts (R^2=0.97-0.99) and serial diluted oocyst DNA elution (R^2=0.95-0.99). These findings suggest that cell line infectivity assay inherently suffers from lowered accuracy, possibly due to the unavoidable losses of oocysts during extensive washing steps. Cell line infectivity assay determined that 400, 500 and 600 MPa HPP treatments led to 2.58, 2.30 and 2.26-log reductions after 3 min, respectively. These findings differ from previous studies as increasing pressure levels did not result in higher reduction levels (p>0.05), possibly due to exacerbated oocysts clumping after membrane damage.

Significance: This study provides relevant information about the suitability of using cell line infectivity assays to validate HPP against *C. parvum* in apple juice, which warrants future studies on developing more suitable assays.

P1-183 Evaluation of a Routine Assay for the Detection of *Cyclospora cayetanensis* in Fresh Produce and Agricultural Water Samples Using Biomérieux GENE-UP Real-Time PCR

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Introduction: Environmental factors and human practices influence the transmission and contamination of fresh produce and agricultural water by *C. cayetanensis*. However, significant gaps in the knowledge of epidemiology, biology, and ecology of *C. cayetanensis* create challenges to traceback of contamination sources.

Purpose: The aim of the project was to develop a routine, efficient, and rapid molecular detection assay for the detection of *C. cayetanensis* in fresh produce and agricultural water.

Methods: The reaction mixture to detect *C. cayetanensis* Mit1C by real-time PCR was developed, and efficiency of the assay was tested using synthetic DNA of *C. cayetanensis* at bioMérieux. The bioMérieux assay kit was further evaluated at FDA for the detection of *C. cayetanensis* in produce and agricultural water samples spiked with low level of the parasite (5 to 6 oocysts) and in clinical samples. DNA was extracted from spiked produce and agricultural water according to the FDA bacteriological analytical manual (BAM) chapters 19b and 19c, respectively. Two µL or 5 µL of DNA/clinical samples were added to 45 µL of the reaction mixture supplied with the kit. The experiment was performed at the following conditions: initial denaturation at 95°C/3 min (one cycle), followed by 40 or 45 cycles of 95°C/ 15 s and 61°C/1 min.

Results: Twenty clinical samples with known Ct values determined by Mit1C qPCR in ABI 7500 Fast were evaluated using the bioMérieux assay kit by Gene Up. A comparable Ct value within 29-38 was detected in the tested clinical samples. Next, the assay kit was investigated using low levels of *C. cayetanensis* to spike produce and agriculture water; 58.3% samples (n=7) were found to be positive within ≤ 38 Ct value.

Significance: The newly developed bioMérieux assay kit could be an effective tool for industry and other organizations for surveillance of this parasite in different foods and environment matrices.

Poster

P1-184 Effects of Environmental Factors on the Persistence of *Cyclospora cayetanensis* Oocysts in Artificially Contaminated Soil and Fresh Herbs Grown under Controlled Conditions

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Undergraduate Student Award Entrant

Introduction: *Cyclospora cayetanensis* outbreaks have affected thousands of persons in the U.S. *Cyclospora* oocysts are highly resistant in the environment and soil may play an important role in the contamination of foods.

Purpose: In this study, *C. cayetanensis* detection in soil and herbs artificially contaminated with the parasite in CONVIRON™ growth chambers was evaluated in two different conditions 1) Under low rainfall/arid conditions with watering once a week and 2) Under wet/humid conditions with watering every two days.

Methods: The soil was inoculated with 400 *C. cayetanensis* oocysts (n=8 spots) and individual leaves of three potted herbs (cilantro, parsley, and basil) were inoculated with 100 oocysts (n=8 leaves/herb). Samples of soil and leaves were collected at 7, 14, 21, 28, 35, 42, 49/52 and 56-days inoculation (dpi). The presence of the parasite in soil was confirmed by concentration via flotation in high density sucrose solutions, DNA extraction and molecular detection by real-time PCR specific for the parasite. The detection of the presence of the parasite in leaves of the herbs followed the Bacteriological Analytical Manual (BAM) chapter 19b method.

Results: In arid/dry conditions, the parasite showed short-lived persistence in soil, with samples only positive for *C. cayetanensis* at 7 dpi. Positive detection results were observed in leaves in the herbs in all the samplings along the study in arid conditions. Under wet/high water content conditions, positive detection was observed at each sampling collection in both soil and herb leaves. Not statistically significant differences in C_T values were observed among samplings (p>0.05).

Significance: The results indicated the short-term persistence of oocysts in soil when herbs are grown in dry conditions. These data will allow FDA to better understand ways of *C. cayetanensis* transmission and to establish control measures to disrupt the cycle of transmission.

P1-185 Exploring the Association Between Parasitic Infection and Climate Parameters in Ethiopia

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Developing Scientist Entrant

Introduction: Intestinal parasitic infections like *Giardia lamblia*, *Entamoeba histolytica*, and soil-transmitted helminths (STH) are estimated to cause a significant portion of the foodborne disease burden, especially in the Global South. Temperature and rainfall can impact parasite survival, and moisture can contribute to larvae movement through the environment, increasing the likelihood of exposure through contaminated food or water sources.

Purpose: The objective of this study was to assess the relationship between laboratory-confirmed parasitic infection and rainfall and temperature in Ethiopia.

Methods: A retrospective epidemiologic study was conducted to estimate prevalence of parasitic infection from stool samples tested from 2018 – 2022 at three hospitals in Ethiopia (Addis Ababa, Gondar, Harar). Weather data from the Ethiopian Meteorological Institute for the same time period were used to assess the relationship between climate variables (monthly rainfall, maximum temperature, minimum temperature) and detection of parasites (*Giardia lamblia*, *Entamoeba histolytica*, STH) in stool samples. Poisson and negative binomial regression models were used to determine the incident rate ratio for parasitic infection at each site.

Results: Of 48,643 samples analyzed, a total of 6,120 samples tested positive for the pathogens of interest across the three sites. Total monthly rainfall was largely not significantly associated with infection, although there was a 0.90% increase in risk of *Giardia lamblia* infection for every millimeter increase in total monthly rainfall. Risk of *Giardia lamblia* infection increased 18.60% and 77.90%, respectively, in Addis Ababa and Harar with each degree increase in monthly maximum temperature. Risk of STH increased 8.20% and decreased 9.30%, respectively, for each degree increase in monthly minimum temperature and maximum monthly temperature in Gondar. Climate variables were not significantly associated with *Entamoeba histolytica* infection at any study sites.

Significance: Results suggest that associations between parasitic infection and climate variables vary geographically in Ethiopia and warrant further study to allocate adequate resources for parasitic infection mitigation.

P1-186 Point-of-Care Lamp-CRISPR/Cas12a of *L. monocytogenes* Using Positive Charge Magnetic Enrichment

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Introduction: The prevention of *Listeria monocytogenes* infections requires the development of a rapid, sensitive, cost-effective, and simple detection method.

Purpose: This study aimed to enhance the sensitivity of LAMP-CRISPR/Cas12a by functionalizing magnetic nanoparticles (MNPs) with tetraethylenepentamine (TEPA) possessing a strong positive charge, enabling the capture and concentration of target pathogens.

Methods: TEPA-functionalized MNPs were synthesized, and their interaction with *L. monocytogenes* was assessed at various pH values using zeta potential measurements and dynamic light scattering. Optimization included TEP-MNP concentration (5–50 µg/mL), reaction time (1–20 min), and magnetic separation time (1–10 min). Additionally, LAMP primers were selected, and the ssDNA reporter concentration (0–1.2 µM) and cleavage times (0–20 min) were optimized for Cas12a-mediated trans-cleavage. Specificity was evaluated on 5 strains of *L. monocytogenes* and 7 strains of non-*L. monocytogenes*. Sensitivity was evaluated using concentrations of *L. monocytogenes* ranging from 2.6×10^0–10^5 CFU/mL(g) in pure culture and enoki mushrooms.

Results: *L. monocytogenes* maintained a negatively charged surface, while TEPA-functionalized MNPs exhibited a strong positive charge due to abundant amine groups covering the entire surface. *L. monocytogenes* with a negative charge facilitated electrostatic adsorption onto TEPA-functionalized MNPs below pH 8.83. TEPA-functionalized MNPs at a concentration of 40 µg/mL effectively captured over 90% of *L. monocytogenes* through a simplified process consisting of a 15 min reaction time and 5 min magnetic separation. The developed method demonstrated a tenfold improvement in sensitivity compared to existing methods. The LAMP-CRISPR/Cas12a biosensor utilizing TEPA-functionalized MNPs achieved detection limits of 10^0 CFU/mL in pure culture and 10^0 CFU/g in enoki mushrooms.

Significance: The combination of TEPA-functionalized MNPs and the LAMP-CRISPR/Cas12a biosensor can be a cost-effective and scalable biosensor for point-of-care testing of foodborne pathogens in the food industry.

Poster

P1-187 Comparison and Verification of Quantitative Analysis for Low Levels of *Listeria monocytogenes* in Cabbage and Culture by Digital PCR, RT-PCR, and Plate Count Method

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Introduction: Digital PCR (dPCR) has emerged as a technology for absolute quantification. Internationally, there are quantitative standards (<100 CFU/g) for *Listeria monocytogenes* in RTE foods, but the culture method is time-consuming. Therefore, it is necessary to compare and verify molecular analytical approaches as alternatives to culture methods.

Purpose: We aimed to examine the feasibility of quantification using dPCR and compare plate counts and qPCR for low levels of *L. monocytogenes* in cabbage and culture.

Methods: *L. monocytogenes* (ATCC 15313) was cultured overnight at 35℃ in TSB. Initially, 6 log CFU/mL of *L. monocytogenes* was used, and then 10-fold serially diluted. 1 mL of each diluent was extracted to obtain DNA. For inoculation, 90 mL of each serial-diluent was added to 10 g of cabbage. After homogenization for 5 min, 1 mL of each mixture was used for DNA extraction. Plate counts were performed on oxford agar and chromogenic agar. DNA was extracted by boiling (95℃, 10 min) and/or by automatic equipment with commercial kit. The extracted DNA was analyzed by dPCR and qPCR, and each data was compared with plate count.

Results: As a result, both dPCR and qPCR quantified 2.81 log CFU/mL of *L. monocytogenes* (1.57-1.65 copies/μL and 35.39-36.25 Ct/copies) in cultures. However, only dPCR could quantify 1.92 log CFU/mL (0.11-0.18 copies/μL). In cabbages, 3.56 log CFU/mL of *L. monocytogenes* was quantified as 1.63-2.20 copies/μL by dPCR and 35.74-36.05 Ct/copies by qPCR. However, more sensitive results could be obtained at 1.56-2.58 log CFU/mL (0.11-0.28 copies/μL) by dPCR than qPCR. Additionally, in the case of boiling extraction of *L. monocytogenes* cultures, both dPCR and qPCR could detect *L. monocytogenes* copies at 4.75 log CFU/m, while 3.75 log CFU/mL was detected only by dPCR.

Significance: Quantification by dPCR was more sensitive than qPCR. However, there were limitations in quantifying low levels of *L. monocytogenes* directly without enrichment.

P1-188 Comparison Study Between the Dry Rehydratable Film and Baird Parker Agar Plate for *Staphylococcus aureus* Enumeration in Selected RTE Products

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Introduction: Recently, regulations on ready-to-eat products have arisen and appropriate detection solutions are required accordingly.

Purpose: We try to find an effective *Staphylococcus aureus* detection method and workflow by comparing the performance and time to result with a challenged or a normal ready-to-eat products.

Methods: Dry Rehydratable Film (DRF; Petrifilm Staph Express Count) Plates and Baird Parker Agar (BPA) plates were inoculated with 2 different frozen RTE food samples containing pure several strains as 4 microbial levels in the validated counting range, respectively. 2 types of colony counters and 3 trained technicians in 2 labs for each microbial levels of DRF and BPA Plates. For confirmation, disk coagulates and MALDI-TOF were used. The spending time for plating and counting was recorded from the first to last inoculation completion/colony counting on one plate.

Results: Two enumeration methods were not equivalent before confirmation. Sampling in two frozen rice products revealed BPA to be insufficiently selective through identification for presumptive *S. aureus*. 60~69% of isolates in high background CFU of sample from BPA were not *S. aureus* while, 0~32% of isolates from BPA were not in low naturally contaminated sample. However, almost of *S. aureus* in DRF plates were recovered from two type of samples properly. Results of productivity study using time check in each experimental step showed that time saving in inoculation and interpretation in DRF plate were about 90% and 17~55%, respectively. Two proposed confirmation methods have essentially good relationship with each other.

Significance: This study will be helpful to select an experiment method for growing RTE products by finding an effective and fast detecting and enumerating *Staphylococcus aureus* compared to the standard method.

P1-189 An Automated Highly Multiplexed PCR Method for *Listeria* Fingerprinting Also Provides Improved Confirmation Rates with Presumptive Environmental Sample Enrichments

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Introduction: Food producers often wish to characterize *Listeria* spp. positive environmental samples for source tracking/trending purposes but manufacturer support for the most common platforms have declined or ended. Whole genome sequencing could provide this characterization, but users have concerns about security and privacy of sequence files. Additionally, cultural confirmation of *Listeria* presumptives, particularly from environmental samples, continues to be a challenge industry wide. The Rheonix *Listeria* PatternAlertTM method can provide both characterization and confirmation directly from presumptive sample enrichments.

Purpose: To evaluate an automated highly multiplexed PCR system for the characterization and molecular confirmation of presumptive environmental sample enrichments in comparison to traditional cultural confirmation.

Methods: Presumptive environmental sample enrichments from a real time PCR screening method (Eurofins Gold Standard Diagnostics' BAC*Gene*™ *Listeria* spp.) were tested by automated highly multiplexed PCR (1.25ml) and with a *Listeria* spp. FDA-BAM-based cultural method. All samples were pre-treated with DNAse to destroy exogeneous DNA prior to real- time PCR.

Results: Of the 68 presumptives tested, only 19 (28 %) were confirmed as *Listeria* by culture. In contrast, the highly multiplexed PCR system detected these same 19 samples plus 10 more (43%). Two unique fingerprint patterns were observed among the detected samples. Interestingly 7/10 presumptives that were detected by automated multiplexed PCR but not by culture were Cq < 30, suggesting *Listeria* was present at high levels in these samples.

Significance: The automated highly multiplexed PCR method showed superior performance to culture: detecting more presumptives, thereby reducing the non-confirming presumptive rate. All samples in the study were pretreated to destroy exogeneous DNA prior to testing by the rapid screening method so dead cell detection is not likely the primary explanation for the poor overall confirmation rate. These results suggest that molecular confirmation using the Rheonix platform, may be a viable alternative to traditional cultural confirmation methods.

P1-190 Rapid Detection of *Listeria* spp. and *L. monocytogenes* Using the Loop-Mediated Isothermal Amplification (LAMP) Assay – Bioluminescent in Liquid, Fresh and Dry Yeast

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Introduction: Yeast is widely applied for fermentation processes in baking, beverage, and other industries. This matrix poses a challenge for pathogen detection due to its high background flora nature. Choosing a rapid and accurate Listeria spp. and L. monocytogenes detection method in yeast samples is important for monitoring effectiveness and for taking action to avoid product contamination.

Purpose: Determine the sensitivity, relative trueness (RT), relative limit of detection (RLOD), and acceptability limit (AL) of the *Listeria* spp. and *L. monocy-*

togenes LAMP-Bioluminescent assay in yeast compared cultural confirmation (ISO 11290-1:2017).

Methods: Yeast samples (n=30) from the Brazilian industry were analyzed by rapid method for *Listeria* and *L. monocytogenes* and compared to the ISO 11290-1:2017 biochemical confirmation. Samples were divided into groups of liquid yeast, fresh yeast and dry yeast (n=10 each). Samples were artificially contaminated at a fractional level using a wild strain of *L. monocytogenes*, adapted to the matrix, spiked in 2 levels, low (n=12, N1=2 CFU/test portion) and high (n=12, N2=16 CFU/test portion). Samples were enriched with Demi Fraser broth (liquid and fresh yeast at 1:10 and dry yeast at 1:20), incubated (37°C/26 hours) and analyzed with LAMP-Bioluminescent assay and by ISO 11290-1:2017. Parameters required by ISO 16140-2:2016 for method comparison were determined.

Results: The alternative LAMP-Bioluminescent assay presented, respectively, a sensitivity, relative trueness and relative limit of detection of 95.8%, 96.7%, 1.16. Acceptability limit evaluated through negative deviation (ND) and positive devations (PD) values revealed ND-PD>3 and ND+PD>6, demonstrating the rapid method is fit for purpose when compared to ISO 11290-1:2017 confirmation according ISO 16140-2:2016 parameters.

Significance: The Neogen® Molecular Detection assay 2 – Listeria and Molecular Detection Assay 2 – Listeria monocytogenes enabled reliable and rapid detection of *Listeria* spp. and L. monocytogenes liquid, fresh and dry yeast.

P1-191 Evaluation of Alternative Sample Preparation and Loop-Mediated Isothermal Amplification (LAMP) Bioluminescent Assay and Comparison Against ISO 11290-1 for Detection of *Listeria monocytogenes* in Frozen Edamame

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Introduction: Food samples from plant origins may contain natural antimicrobial compounds and background microflora. Microbiological testing of plant-based foods requires appropriate sample preparation procedures to enable recovery and detection of pathogens from these matrices. Major changes to analytical procedures should be validated to ensure that low levels of pathogens can still be detected.

Purpose: This study evaluated alternative sample preparation and loop-mediated isothermal amplification (LAMP) bioluminescent assay for the detection of *Listeria monocytogenes* in pooled 125-g test portions of frozen edamame and compared its performance against reference method ISO 11290-1.

Methods: The method comparison study was performed according to ISO 16140-2 using frozen edamame as a test matrix. Different sample sizes (25-g and 125-g) of three different commercial frozen edamame products were spiked with *Listeria monocytogenes* ATCC 49594 at different levels ranging from <0.1 to 20 CFU per test portion. Enrichment was performed using Demi-Fraser Broth at 1:20 dilution and incubation at 30ºC for 24-30 h for 125-g samples, and at 1:10 dilution and incubation at 30ºC for 25h for 25-g samples, respectively, followed by secondary enrichment in Fraser Broth. Analyses for *L. monocytogenes* were performed using both LAMP assay and biochemical confirmation.

Results: The limit of detection (LOD_{50}) for *L. monocytogenes* in 125-g sample was 0.939 CFU using LAMP method with primary enrichment and 0.472 CFU with secondary enrichment, respectively. LOD_{50} for 25-g sample using ISO 11290-1 procedure was 0.239 CFU. When comparing the alternative pooled size (125 g) enriched at 1:20 dilution and with secondary enrichment against the reference 25-g method, the RLOD value was 1.97 (<2.5), indicating equivalency between the two procedures.

Significance: Results showed that LAMP-based assay can be an effective method for rapid detection of *L. monocytogenes* in frozen edamame. Successful detection of this pathogen in 125-g frozen edamame can be achieved with higher sample dilution (1:20) and with secondary enrichment.

P1-192 Biotyping of an Internal Library of *Listeria* Species Using the Rheonix Listeria PatternAlert™ Assay

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Introduction: Persistent *Listeria* that find a harborage site in food facilities can significantly increase the risk of broader contamination. Presence/absence screening alone cannot identify recurring strains. Biotyping of isolates may allow food manufacturers to better risk assess the possibility of resident strains compared to transient strains in their environment.

Purpose: An internal collection of *Listeria* species consists of samples isolated from food or environmental sources or obtained directly from strain collection suppliers. These isolates can be leveraged to create a reference library for future identification of similar biotypes of *Listeria* species.

Methods: This study evaluated the performance of the assay on the *Encompass*™ *Optimum* Workstation with the Listeria PatternAlert™ Assay Kit[Ls] to characterize an internal library of over 50 *Listeria* species which were wild-type or commercially obtained. *Listeria monocytogenes* (n=30) and *Listeria innocua* (n=18) and other *Listeria* species including *grayii*, *welshimeri*, *seeligeri*, and *ivanovi* were evaluated. The workstation performs automated PCR on isolated samples to detect unique sequences using 15 targets on a low-density DNA microarray, and assigns different patterns based on the presence or absence of *Listeria* locus detected.

Results: Of the over 50 samples of *Listeria* isolates tested, 20 different patterns emerged. *L. monocytogenes* species shared the same distinct target across 13 patterns, correlating to an environmental origin or food type. *L. innocua* had 4 distinct patterns, the majority of which shared one target. Sometimes, a single specific target was assigned for a species, such as for *L. grayii*. This allows for discrimination between different strain types.

Significance: Understanding the genetic diversity of internal *Listeria* species enables faster, more informative assessments of unknown samples, ensuring earlier detection and intervention for food safety. This system may provide a tool to support environmental monitoring investigations and risk assessments regarding resident or transient *Listeria* strains within an environment.

P1-193 Understanding Non-Confirming Presumptives in Environmental *Listeria* Testing

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Introduction: Molecular methods have revolutionized pathogen testing, but samples that test presumptive positive are typically confirmed using cultural methods. As the sensitivity of molecular methods continue to improve, the frequency of presumptive results that fail to culturally confirm has increased, though the factors underlying these lower confirmation rates are poorly defined or quantified.

Purpose: This study was designed to determine the main cause(s) of presumptive *Listeria* spp. environmental samples that fail to confirm by culture.

Methods: A total of 634 environmental sponges submitted over a five-month period were tested for *Listeria* spp. using commercial real-time PCR and end-point assays. DNase treatment was employed prior to cell lysis to eliminate free DNA and reduce the possibility of false positives from dead cell detection. All presumptive positive samples were confirmed by the FDA-BAM culture method. Samples that did not confirm using the standard BAM method were subjected to further analysis, including a secondary enrichment, additional plating media and increased plating volumes. Enrichments were also sequenced to verify if *Listeria* was present.

Results: A total of 66 samples were presumptive for *Listeria* spp. by one or both of the PCR platforms used. Of these 66 presumptive samples, fifteen were unable to be confirmed by standard culture methods. The addition of secondary enrichments, plating on different selective and differential agars, and increased plating volumes all failed to recover *Listeria* in any of these fifteen samples. Whole genome sequencing of the enrichments demonstrated that *Listeria* DNA was present in all fifteen samples. Because DNase was used prior to lysing each of these samples, suggesting that viable *Listeria* was present in the samples, despite the cultural methods failing to recover the organism.

Significance: These data suggest that the results of molecular methods for pathogen testing may be more reliable than cultural methods.

P1-194 Evaluation of a Proprietary *Listeria* Selective Media for Recovery of Low Levels of *Listeria spp.* from Sponges Hydrated with Wide Spectrum Neutralizer (WSN) and Letheen Followed by Molecular Detection Using Loop-Mediated Isothermal DNA Amplification

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Introduction: Environmental monitoring of *Listeria* is an important verification activity to demonstrate that sanitation programs are under control and to identify potential niches of *Listeria* that can pose a risk in contamination of ready to eat foods. Considering the fast-paced environment in food production, having rapid tools for detection of *Listeria* would enable faster assessment of the status of the food manufacturing environment. However, *Listeria* often shows slower growth and requires enrichment times of up to 48h for recovery.

Objective: To evaluate the use of a proprietary enrichment (LESS-plus) to recover *Listeria* from WSN and Letheen sponges followed by LAMP detection and to compare its performance when sponges are evaluated following ISO 11290-1:2017.

Method: A total of 50 sponges containing WSN broth and 50 sponges containing Letheen were used to collect samples in a manufacturing facility. After collection, sponges were shipped to the laboratory and spiked with approximately 1CFU (N=40-low) and 5CFU (N=10-high) of *Listeria monocytogenes* (ATCC49594) to achieve fractional recovery. Sponges were refrigerated for 96h. For each set of 25 sponges (20-low and 5-high), sponges were enriched with 100mL of LESS+ or 100mL of Demi-Fraser and incubated at 37°C. After enrichment, all samples were analyzed with LAMP for detection of *Listeria* after 16-24h and subjected to culture confirmation. Study was repeated, but spiking sponges with *Enterococcus faecalis* (10 CFU) as competitive organism and enriching with 60mL of LESS-plus for the alternative method.

Results: The relative limit of detection was calculated for the two methods following AOAC guidelines, the p-value when comparing recovering of *Listeria* >0.05 for the 16-24h range when using WSN or Letheen. When comparing recovery between WSN and Letheen, WSN showed significant larger recovery (p<0.05) than Letheen.

Significance: The evaluated proprietary media may offer faster time to result for Listeria environmental monitoring and verification of sanitation programs.

P1-195 Decreasing the Confirmation Time for *Salmonella* and *Listeria* Using an Alternative Procedure Coupled with Hygiena's BAX® System

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Introduction: Culture procedures for confirming a presumptive positive screening result can take several days to complete. After the initial positive result, the target organism is isolated using secondary selective enrichments and/or plating agars to obtain suspect colonies. Then, further biochemical tests and serology are required for identification.

Purpose: The purpose of these studies was to evaluate an alternative confirmation protocol for *Salmonella* and *Listeria* initiated directly from the primary enrichment to confirm presumptive positive results with a quicker turn-around time.

Methods: Four matrices, including environmental sponges, pasteurized liquid egg whites and whole eggs, and dried whole egg powder, were enriched according to validated protocols for *Salmonella* and *Listeria*. After incubation, aliquots were inoculated with various *Salmonella* or *Listeria* cultures at 10^4 CFU/mL, 10^5 CFU/mL and 10^6 CFU/mL. Samples were confirmed following two procedures: the alternative confirmation procedure with additional colony testing on an AOAC-certified real-time PCR method, and the traditional confirmation procedures in the USDA FSIS reference methods.

Results: For *Salmonella*, both the alternative and traditional confirmation procedures produced typical colonies on XLD, DMLIA and Brilliance™ *Salmonella* for all three inoculation levels and matrices. Similarly, for *Listeria*, the alternative and traditional confirmation procedures produced typical colonies on MOX and PALCAM for all three inoculation levels and matrices. *Salmonella* and *Listeria* colonies were additionally tested using real-time PCR as a rapid molecular confirmation. All colony suspensions returned positive results.

Significance: Overall, the results demonstrate equivalent performance between the alternative confirmation procedures and the USDA-FSIS reference culture methods to isolate *Salmonella* and *Listeria*. Furthermore, colony testing with the BAX® System can shorten the confirmation time.

P1-196 Matrix Verification of 25 g of Soppressata for the Detection of Shiga Toxin-Producing *E. coli* (STEC) Using Hygiena's BAX® System Real-Time PCR Assay

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Introduction: Ready-to-eat (RTE) meat and poultry products such as hot dogs and cold cuts are in an edible form that does not require any additional preparation by the consumer. These products are processed by the manufacturer by cooking, smoking, fermenting, or other lethality treatment to ensure product safety. Despite these processing techniques, RTE foods are still at risk for contamination and are often implicated in product recalls.

Purpose: This study was designed to verify the use of a real-time PCR assay for the detection of non-O157 STEC organisms compared to the USDA FSIS reference methods in unpaired samples of soppressata.

Methods: In two separate matrix studies, soppressata was inoculated with either *E. coli* O121 or *E. coli* O103 to create a low-level spike expected to yield fractional positive (25 to 75%) results. Additional samples were left uninoculated as controls. Following a 4 °C equilibration period, samples (25 g) for the test method (n=12) and reference method (n=12) were enriched according to their respective procedures and confirmed following the USDA MLG Chapter 5C.03.

Results: For the test method, real-time PCR detected 5/6 positives for *E. coli* O121 and 4/6 positives for *E. coli* O103. All uninoculated controls were negative, and all results were identical to culture. The probability of detection and difference in POD (POD/dPOD) was used to compare the results between the test and reference method, demonstrating no significant difference for either organism.

Significance: The results of this study demonstrate that the BAX® System Real-Time STEC Suite is accurate and reliable for the detection of Shiga Toxin-Producing *E. coli* in 25 g samples of soppressata, statistically equivalent to culture.

P1-197 Matrix Validation of 30 g Test Portions of Soil for the Detection of *E. coli* O157:H7, *Salmonella* and *Listeria* Using Hygiena's BAX® System

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Introduction: Soil health plays a fundamental role in crop health and production. Good soil quality provides essential elements for crop growth and yield as well as a diverse microbial community that aids in regulating deleterious organisms. Shifts in weather and climate factors can expose agroecosystems to harmful pathogens and put food crops at high risk.

Purpose: This study was designed to validate soil impacted by heavy storms for the detection of *E. coli* O157:H7, *Salmonella* and *Listeria* using a rapid, real-time PCR-based method.

Methods: Two unpaired matrix studies were performed following the technical guidelines in AOAC INTERNATIONAL Official Methods of Analysis Appendix J. Soil was co-inoculated with *E. coli* O157:H7 and *Salmonella* Typhimurium (study 1), or *Listeria* only (study 2) at a low fractional level and at a high level. Samples were equilibrated at 4°C for approximately 72 hours. Test method samples (30 g) were enriched and tested by real-time PCR, while reference method samples (30 g) were enriched and confirmed according to their respective procedures in the EPA and FDA reference methods.

Results: Test method samples analyzed by real-time PCR detected 6/20 positives for *E. coli* O157:H7, 7/20 positives for *Salmonella* and 10/20 positives for *Listeria* in the low inoculum level samples. All high-inoculated samples were positive. These results were in complete agreement with culture. When compared to the reference method using the probability of detection (POD), there was no significant difference for any target organism.

Significance: This study shows that the BAX® System Real-Time PCR assays are specific, sensitive and accurate for the detection of *E. coli* O157:H7, *Salmonella* and *Listeria* in 30 g samples of soil.

P1-198 Microbial Verification of a Molecular Technique for *Salmonella* spp. Detection on Pork Meat, Pork Liver, Rectal Swabs and Feces According to ISO 16140-3 on Colombia

Ruth Dallos

Neogen Food Safety, Bogotá, Chico Norte, Colombia

Introduction: Rapid and accurate detection of pathogenic microorganisms detects risks before jeopardizing consumer health. Molecular detection methods provide correct and faster results than culture detection methods. User labs must demonstrate that a validated method performs according to the method's specifications and is fit for its intended purpose.

Objective: To verify the Molecular Detection Assay 2 – *Salmonella* on pork meat, pork liver, fecal swabs and fecal material for – PorkColombia Laboratory, in Bogota, Colombia.

Methods: Four matrices were evaluated: pork meat, pork liver, rectal swabs, and fecal material. Samples were obtained from farms and a butcher shop in the department Cundinamarca in Colombia. Samples were transported under refrigerated conditions to the laboratory PorkColombia in Bogota, Colombia. Samples were added with enrichment broth according to the AFNOR Validation 3M 01/16-11/16. Seven samples per matrix were added with an average of 5 cells of an overnight *Salmonella* culture (*Salmonella tyhpimurium* ATCC 14024), following the ISO 16140-3 instructions (protocol 3). After enrichment for the detection/identification of *Salmonella* spp., the MDS-3M Salmonella-2® Molecular Detection System was used by two different technicians. One un-spiked sample was used as a control for each matrix.

Results: Six, seven, six and seven out of the seven spiked samples were reported as positive for pork meat, pork liver, fecal swabs, and fecal material respectively by the Molecular detection method. All the negative samples were reported as negative. According to ISO 16140-3 matrices can be considered as verified.

Significance: The user PorkColombia Laboratory successfully verified matrices tested and can use the method with confidence.

P1-199 Verification of a Rapid Method to Enumerate Microbial Indicator on Several Matrices on Ecuador

Ruth Dallos

Neogen Food Safety, Bogotá, Chico Norte, Colombia

Introduction: Before implementing an alternative method in the laboratory, performance and fit for purpose must be evaluated. ISO 16140-3 describes a standard and globally recognized protocol to verify. Methods must be validated and then verified. The verification step must be performed by the lab user, using the local matrices, and comparing with key parameters included in the ISO standard.

Purpose: To verify a quantitative rapid method for microbial indicators on five different food categories and environmental samples in a laboratory located on Manta, Ecuador.

Methods: Eight Petrifilm tests were evaluated: Rapid Aerobic Count, Aerobic Count, Coliform Count, *E. coli/* Coliform Count, Rapid *E. coli/* Coliform Count, Staph Express, Enterobacteriaceae Count and Rapid Yeast and mold Count. Ten matrices belonging to five food categories and one non-food category were evaluated. Ten-gram samples were used. Each sample were added with 90 mL of Butterfield Phosphate Buffer. Fresh overnight culture (*E. coli* ATCC 25992, *S. aureus* ATCC 25923, *S. cerevisiae* ATCC 9763, *K. aerogenes* ATCC 13048, *Salmonella* Typhimurium ATCC 14028) was used to contaminate at different levels (2-800 CFU/sample). Each sample was mechanically homogenized and ten-fold diluted. One milliliter aliquot was transferred to Petrifilm and subsequently was incubated following AOAC-OMA validation. Replicates, Reproducibility standard deviation (SIR) and Estimated bias (eBias) were calculated following ISO 16140-3 scheme.

Results: Implementation verification is accepted for all the tests and categories tested since every SIR value was lower than two times those obtained during validation: 0.204 versus 0.352 on average. For the food item verification step, all the eBias was lower than 0.5 log for all the combinations. All the Petrifilm assays can be considered as verified since they meet both criteria.

Significance: Petrifilm methods were fully verified for the five food categories and one non-food category tested according to ISO 16140-3.

P1-200 Optimization of Pre-Enrichment and Screening Methods for the Detection of *Salmonella enterica* in Meat Analog Products

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❖ ***Developing Scientist Entrant***

Introduction: Meat analogs, popular for health, sustainability, and ethical reasons, have the potential for *Salmonella enterica* contamination. The current Food and Drug Administration (FDA) regulatory methods for *Salmonella* detection in meat analogs follow the Bacteriological Analytical Manual (BAM) and are time-consuming and costly, requiring pre-enrichment using lactose broth with a surfactant (e.g., Triton X-100).

Purpose: This study aimed to optimize pre-enrichment and screening methods by assessing modified techniques against the FDA reference method.

Methods: Meat analog samples were inoculated with *Salmonella* at three levels according to FDA validation guidelines: (1) fractional inoculated level (50% ± 25% of tests positive in at least one method), (2) +1 log inoculated level (100% positive results in at least one method), and (3) uninoculated control. The FDA reference method using lactose broth with Triton X-100 (LB-T) for pre-enrichment followed by culture confirmation was compared to alternative pre-enrichment methods using lactose broth (LB) or buffered peptone water (BPW). Additionally, the current screening method (VIDAS) was compared to rapid screening methods (LAMP and qPCR).

Results: Pre-enrichment with BPW exhibited the highest positivity rate for culture-based confirmation (24/25; 96%) compared to LB-T (23/25; 92%) and LB (22/25; 88%). Regarding screening methods, qPCR yielded the overall highest positivity rate (67/75; 89%) compared to LAMP (64/75; 85%) and VIDAS (63/75; 84%). No statistical difference ($p>0.05$) was found among the three broths or the three screening methods based on a Pairwise Test of Equality of Proportions.

Significance: These results suggest that surfactants are unnecessary for the detection of *Salmonella* in meat analogs. Furthermore, these findings suggest that LAMP and qPCR are effective screening alternatives to VIDAS. The adoption of the modified, more efficient methods tested in this study will reduce the time and cost associated with *Salmonella* detection in meat analogs.

P1-201 Performance Evaluation of Fluorescence Resonance Energy Transfer-based Real-Time PCR for Detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium from Poultry Carcass Rinsates

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Introduction: European and some South American countries now require raw poultry products that test positive for *Salmonella* spp. to also be screened for the presence of *Salmonella* Enteritidis and *Salmonella* Typhimurium. GENE-UP® *S.* Enteritidis and Typhimurium duplex assay (SE ST) is a real-time PCR assay that utilizes FRET hybridization chemistry to provide simultaneous multi-target detection.

Purpose: The alternative method was evaluated for its ability to detect low levels of *S.* Enteritidis and *S.* Typhimurium in chicken carcass rinsates.

Methods: For the verification study, chicken carcasses were rinsed with 400mL nBPW. A 30mL aliquot of the rinse was combined with 30mL of BPW and incubated for 18 hours at 42°C. Artificially contaminated samples were spiked with ~1 CFU/test portion and non-contaminated samples were also evaluated. All samples were screened using the alternative rapid method and the MLG 4.14 reference method was used to confirm all samples for the detection of *S.* Enteritidis and *S*. Typhimurium in the carcass rinsates.

Results: The results showed 100% agreement between the alternative rapid screening method and the MLG 4.14 reference confirmation method for the fifteen replicates evaluated. Further confirmation was performed by running a colony isolated on selective media back through the PCR assay.

Significance: GENE-UP SE ST duplex assay is a rapid, real-time PCR method for the simultaneous detection of *S.* Enteritidis and *S*. Typhimurium in raw poultry products. The ability to multiplex these assays helps streamline the testing requirements for poultry producers resulting in the ability to make quicker decisions.

P1-202 Validation of the GENE-UP® EHEC Method Using the eGENE-UP® EASYPREP Solution for the Detection of STEC in MicroTally® Cloths

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Introduction: In 2023, the USDA FSIS issued a directive updating the N60 sampling guidance for testing beef trim for the Top 7 STEC to incorporate MicroTally® sampling cloths as its preferred method. As the industry has moved to adopt this directive, it is imperative to ensure that the methods used by testing laboratories are validated for the new swab. The GENE-UP® EHEC assay is a real-time PCR molecular detection method that utilizes FRET technology for the rapid detection of the Top 7 STEC. The method is an AOAC *Official Method of Analysis*SM (2020.06) for the detection of STEC using the new sampling cloths in as little as 8 hours of enrichment. To reduce the enrichment time further, the method was evaluated using an automated preparation protocol, the eGENE-UP® EASYPREP Solution after 4 hours of enrichment.

Purpose*:* To conduct a method modification study of the candidate method with the addition of an automated preparation protocol for the detection of STEC from carcass sampling cloths.

Methods*:* The candidate method was evaluated according to AOAC Appendix J validation guidelines. An unpaired matrix study of the carcass sampling cloth in BPW after 4 hours of enrichment was performed. Test portions were confirmed following procedures in the MLG 5C.03 reference method and an alternative immunocapture detection method using the VIDAS® instrument.

Results*:* The results of the study indicated no differences between presumptive and confirmed results for the candidate method using the automated preparation protocol or traditional lysis protocol. There were no statistically significant differences observed between the candidate method and the MLG reference method.

Significance: The candidate method with automated preparation can reduce the enrichment time needed for STEC detection to four hours for the new carcass sampling cloths.

P1-203 A Rapid Culture-Independent Detection Method of *Salmonella* spp. in Poultry Carcass and Feed Utilizing Immunomagnetic Separation, Whole Genome Amplification, and LAMP

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✦ ***Undergraduate Student Award Entrant***

Introduction: Efficient and timely detection of foodborne pathogens, particularly *Salmonella* in the poultry industry, is crucial for preventing outbreaks. However, traditional culture-based methods are often hindered by prolonged processing times. Therefore, rapid method alternatives are necessary.

Purpose: This study aims to develop a rapid culture-independent method for detecting *Salmonella* in poultry carcasses and feed, utilizing immunomagnetic separation and whole genome amplification (IMS-WGA) followed by Loop-Mediated Isothermal Amplification (LAMP), with the primary objective of reducing detection time while maintaining sensitivity for on-site applications.

Methods: *Salmonella* inoculations were performed to 25g of carcass and feed samples from 10^5 to 1 CFU/g and homogenized with 225 mL of Buffered Peptone Water (BPW). IMS was used to separate pathogens from poultry carcasses and feed samples selectively. To overcome the challenges associated with low bacterial loads, we utilized Multiple Displacement Amplification, a kind of WGA, which bypasses the need for an enrichment process. The amplified DNA was subjected to LAMP for rapid and sensitive detection. Both fluorescence-based real-time and pH-based colorimetric methods were applied and compared.

Results: The IMS-WGA-LAMP was successful in detecting 10 CFU/g of *Salmonella* in spiked poultry carcass samples in 80% (8/10) and feed samples in 70% (7/10) using the real-time LAMP method, while IMS-LAMP and direct LAMP or PCR were unable to detect it. The average time to threshold (Tt) value was 23.3 (0.38) for carcass and 23.7 (0.42) for feed, as determined by mean (SD). The colorimetric method exhibited a limit of detection (LOD) of 10^3 CFU/g for both samples. Assay inclusivity and exclusivity were validated using 26 strains of 19 serotypes of *Salmonella* spp. and 6 strains of non-*Salmonella* species.

Significance: The IMS-WGA-LAMP method has a turnaround time of 5 hours and do not require complex appliances, making it a valuable tool for on-site rapid and sensitive detection method for *Salmonella* in food safety.

P1-204 Verification of GENE-UP QUANT *Salmonella* Method in Raw Poultry and Carcass Rinsate

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Introduction: *Salmonella* spp. is a major pathogenic bacterium for the poultry industry. Quantitative detection of *Salmonella* spp. is a new trend for traceability of survival of *Salmonella* during production processes. There are many methods for detecting quantitative *Salmonella* spp. that include standard method and alternative method, for example GENE-UP QUANT *Salmonella.* According to ISO17025, alternative method is an option for accredited laboratories. However, method verification is necessary for the laboratory to verify the method before using routinely.

Purpose: To evaluate the performance of GENE-UP QUANT Salmonella by following ISO 16140-3: 2021.

Methods: To verify the accuracy of the method, they inoculated 3 levels of *Salmonella* Typhimurium in 40 samples, then performed testing by GENE-UP QUANT *Salmonella* method and performed actual count of inoculum level by pour plate technique. Accuracy of the method was reported by calculation of absolute difference log CFU between GENE-UP QUANT *Salmonella* results and actual count of inoculum level.

For interlaboratory reproducibility standard deviation (S_{IR}) determination, 20 samples were spiked with three levels of *Salmonella* Typhimurium and testing was performed by following GENE-UP QUANT *Salmonella* method. For estimate bias (eBias) determination, there were eight total samples that included negative control and three levels of *Salmonella* Typhimurium, with two samples of each inoculum level.

Results: GENE-UP QUANT *Salmonella* is a "true count" method as the result of difference log between GENE-UP QUANT *Salmonella* results and inoculum level were lower than 0.5 logCFU and GENE-UP QUANT *Salmonella* was not significant different from actual count of inoculum level at 95% confidence level (P 0.64 > 0.05). GENE-UP QUANT *Salmonella* has a good accuracy and precision as the results of S_{IR} value was 0.129 and eBias of three inoculum levels were lower than 0.5 logCFU.

Significance: The percentage of accuracy of GENE-UP QUANT *Salmonella* was at 100% and P 0.64 > 0.05**.** SIR and eBias were within acceptance criteria following ISO 16140-3: 2021.

Poster

P1-205 Novel Loop Mediated DNA Amplification (LAMP) Based Bioluminescent Assays for the Detection of *Salmonella* Enteritidis and *Salmonella* Typhimurium, Including the Monophasic Variant *Salmonella enterica* I 4,[5],12:i:-

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Introduction: The traditional phenotypic method to determine *Salmonella* serotype is laborious. Quick and easy screens for serotypes of particular interest are highly desirable for the poultry industry. *S* Enteritidis and *S*. Typhimurium are two of the most common serovars isolated from salmonellosis outbreaks resulting from poultry products consumption. Here we describe novel rapid molecular-based detection assays to specifically identify these serotypes.

Purpose: To create and demonstrate the performance of two new Molecular Detection System assays (MDA2-SE/ST) for detection of *Salmonella* Enteritidis (SE) and *Salmonella* Typhimurium (ST), including the ST monophasic variant I 4,[5],12:i:-.

Method: Assay specificity was evaluated using cultures of 44 (SE) or 59 (ST) inclusive isolates, and 217 (SE) or 202 (ST) exclusive serovars. All isolates were whole genome sequenced to confirm serotype. Extensive in-silico and reagent pellet testing was conducted during LAMP assay design to confirm serotype specificity. The assay parameters were tuned and evaluated by amplifying a dilution series of target DNA at defined concentrations. Assay sensitivity was tested with post enrichment spikes at 5 levels (total of 15 nBPW chicken carcass rinses and 30 raw ground chicken samples).

Results: The two new LAMP assays are able to positively detect SE and ST (including I 4,[5],12:i:-). For SE, 44/44 inclusives were positively identified and none of the 217 exclusive cultures were detected. For ST, 59/59 inclusives were positively identified and none of the 202 exclusive cultures were detected. The assay kinetics are comparable to other commercial LAMP-BART assays. From ground chicken and carcass rinse enrichments, the sensitivity of both assays was comparable to the Neogen Molecular Detection Assay 2 - *Salmonella*.

Significance: These new assays have high specificity, accuracy, and sensitivity and can be used reliably to rapidly screen for SE and ST in select poultry products.

P1-206 Novel *Listeria* spp. Detection Approach for Environmental Monitoring Program

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Introduction: *Listeria* is ubiquitous, which poses a continued risk of being introduced from the processing environment into the food chain. An effective *Listeria* environmental monitoring program (EMP) is critical to ensure food safety. Herein, we describe a novel no-enrichment approach for the detection of *Listeria* from environmental surfaces using sponge samplers, with time to result in less than one-hour.

Purpose: To evaluate the performance of ANSR *Listeria* Right Now for environmental sponge test (LRN-Sponge) compared to the FDA BAM *Listeria* detection method.

Methods: Two sets of twenty (20) replicate 4 in. x 4 in. stainless-steel (SS) surface areas were inoculated with *L. monocytogenes* and *E. faecalis* (at 10 times higher level) as background organism, with the intention of producing a fractional *Listeria* positive data set for both LRN-Sponge and reference methods. Five (5) replicate areas were inoculated with both strains at higher levels, and five (5) replicate areas received diluent only as uninoculated controls for each method. For additional verification, the same cell-lysates containing RNA from LRN-Sponge samples were also tested by an independent RT q-PCR *Listeria* method. A statistical model measuring differences of Probability of detection (dPOD) and 95% confidence-interval of dPOD were calculated to compare the two detection methods.

Results: The dPOD and 95% confidence-interval analysis showed there was no significant difference between the LRN-Sponge and reference methods for detection of *Listeria* from the SS surfaces. When the samples from LRN-Sponge were also tested with the independent RT q-PCR assay, there was no significant difference between LRN-Sponge and RT q-PCR results based on dPOD and 95% confidence interval calculations.

Significance: No enrichment, sensitive and rapid detection of *Listeria* from environmental samples in less than an hour is a major advancement in implementing an effective *Listeria* EMP, allowing quick detection of presumptive-positive areas and implementation of immediate corrective action.

P1-207 Validation of the Neogen® Molecular Detection Assay 2 – *Salmonella* Enteritidis/*Salmonella* Typhimurium Method for Specific Detection of *Salmonella enterica* ser. Enteritidis and *Salmonella enterica* ser. Typhimurium in Chicken Carcass Rinse, Raw Ground Chicken and Cooked Breaded Chicken

Quynh-Nhi Le[1], Toni Bartling[2], Mark Mozola[3], Cynthia Zook[2], Christina Barnes[2], Brooke Roman[1], Dr. Preetha Biswas[1], Susan Noe[2] and Robert Donofrio[1]

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Introduction: The Neogen® Molecular Detection Assay 2 – *Salmonella* Enteritidis/*Salmonella* Typhimurium (MDA2-SE/ST) method is a rapid, nucleic acid amplification-based test for specific detection of *Salmonella enterica* ser. Enteritidis (SE) and *Salmonella enterica* ser. Typhimurium (ST), including the ST monophasic variant *Salmonella enterica* I 4,[5],12:i:-, in select poultry samples. Results for SE and ST are generated separately.

Purpose: To validate the rapid molecular method for detection of SE and ST in chicken carcass rinse (25g & 325g), raw ground chicken (325g), and cooked breaded chicken (25g) for AOAC *Performance Tested Methods*℠ certification.

***Methods*:** The study consisted of inclusivity/exclusivity testing and independent laboratory testing of chicken carcass rinse, raw ground chicken, and cooked breaded chicken using inoculated matrices. Chicken carcass rinse and fresh ground chicken were compared to the USDA/FSIS MLG 4.14 reference method while cooked breaded chicken and raw ground chicken were compared to the ISO 6579-1:2017 reference method.

***Results*:** In inclusivity testing, all 50 SE strains produced positive results in the SE assay and negative results in the ST assay. Fifty-three ST strains (including the monophasic variant) produced positive results in the ST assay and negative results in the SE assay. Thirty-five exclusivity strains included multiple non-SE group D_1 *Salmonella* serovars, multiple non-ST group B serovars, *Salmonella* spp. from other somatic groups, and other *Enterobacteriaceae* produced negative results in both assays. In matrix testing, results for the candidate and reference methods were in complete agreement for all matrices evaluated.

Significance: Development of the MDS2-SE/ST method addresses increasing worldwide interest in specific detection of two *Salmonella* serovars prevalent in poultry environments as well as in human illness. Further, use of the test reduces the need for laborious conventional serotyping.

P1-208 Development of a Laboratory Procedure to Produce Highly Injured *Salmonella* spp. on Stainless-Steel Coupons for Efficacy Testing of Environmental Sampling Devices

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Introduction: Monitoring the production environment for pathogenic microorganisms is a key intervention to keep food safe. Studies to assess the effectiveness of sampling devices typically use artificially contaminated surfaces such as stainless-steel coupons; however, there is no standard preparation method for these coupons.

Purpose: A study was undertaken to develop a protocol that can be utilized by all laboratories interested in assessing the performance of sampling devices.

Methods: Different concentrations of *Salmonella enterica* serotype Typhimurium were spotted onto 80 sterile stainless-steel coupons, dried in a biosafety cabinet for 1 hour, and transferred to an incubator at 25°C for 72 hours. Coupons were sampled using EZ Reach™ Sponge Samplers. Viable *Salmonella*

were detected using a culture method employing BPW as a pre-enrichment broth, RV as a selective broth, and XLD for selective plating.

Results: Predictable die-off of the spotted cells occurred when the average relative humidity during the 72-hour hold period was reduced and held in the range of 8-16%. The amount of humidity in the chamber was controlled with freshly activated silica gel desiccant spaced throughout the chamber. A correlation of R^2 = -0.83 was seen between the average relative humidity and the rate of successful recovery of organism. To achieve the optimal humidity, there should be an overall 3.3 kg of desiccant per 0.16 m^3 of chamber volume.

Significance: Environmental monitoring programs are only as good as the materials and methods used to collect, process, and detect microorganisms after surface sampling. This laboratory procedure affords the opportunity to conduct meaningful efficacy studies with environmental sampling devices using stainless-steel coupons with predictable levels of highly injured bacteria to produce fractional positive results.

P1-209 Validation of the Hygiena® foodproof® *Salmonella* plus *Cronobacter* Detection LyoKit Compared to ISO Reference Methods for Infant Cereals, Infant Formula With or Without Probiotics and Ingredients, and Production Environmental Samples

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Introduction: Infections of infants with *Cronobacter* and *Salmonella* species are linked to the consumption of contaminated powdered infant formula (PIF). Therefore, regulatory agencies require screening for these two pathogens throughout the entire production process of PIF.

Purpose: This study evaluated the foodproof *Salmonella* plus *Cronobacter* Detection LyoKit combined with different DNA extraction methods compared to ISO 6579-1 and ISO 22964 standards according to the requirements of DIN EN ISO 16140-2:2016 and the AFNOR Certification technical rules.

Methods: For the method comparison part of this AFNOR Certification validation study, sensitivity, relative level of detection (RLOD) and specificity studies were conducted by ADRIA Développement. For the sensitivity study, 141 (*Salmonella* target) and 137 (*Cronobacter* target) uncontaminated and artificially contaminated samples of probiotic and non-probiotic infant formula, ingredients (375 g) and production environmental samples (200 g and surfaces) were enriched in buffered peptone water (1:10 dilution; with 10 mg/L vancomycin for probiotic-containing samples) and incubated for 16-20 hours at 37 °C. Following incubation, DNA extraction was performed using the foodproof® StarPrep Three Kit and the BAX® Prep Gram-Negative Lysis Kit, followed by real-time PCR analysis. In addition, 60 samples per target were analyzed by the alternative and the reference methods to determine LOD_{50} and RLOD values. Specificity panels including 100 *Salmonella* and 50 *Cronobacter* strains and 30 non-target strains were evaluated to ensure inclusivity and exclusivity of PCR targets.

Results: The method described successfully yielded results comparable to the reference methods. The sensitivity and RLOD results met the acceptability limits for both targets. Specificity studies produce 100% inclusivity for 150 target strains with 100% exclusivity of 30 non-target strains for each target.

Significance: This validation provides the infant formula industry with a real-time PCR method that simultaneously detects *Salmonella* and *Cronobacter* species from one enrichment culture and in only one PCR reaction.

P1-210 Validation of a *Salmonella* Loop-Mediated Isothermal Amplification Assay in 27 Human and Animal Food Matrices of 9 ISO Food Categories

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Introduction: Improvement in *Salmonella* screening methods greatly enhances the efficiency of U.S. Food and Drug Administration (FDA) and state laboratories' food safety testing programs.

Purpose: This study aimed at extending an FDA-validated *Salmonella* loop-mediated isothermal amplification (LAMP) screening assay to 27 human and animal food matrices through an FDA Laboratory Flexible Funding Model (LFFM) collaboration with nine state laboratories. These 27 matrices represented nine ISO food categories (chocolate, bakery products and confectionary; dried cereals, fruits, nuts, seeds and vegetables; eggs and egg products [derivatives]; environmental samples [food and feed production]; fish and seafood products; fresh produce and fruits; infant formula and infant cereals; milk and dairy products; and pet food and animal feed).

Methods: From July 2022 to December 2023, participating laboratories received 30 blinded test portions per matrix spiked with low, high, and uninoculated levels of *Salmonella*. All test portions were prepared for *Salmonella* isolation, according to FDA's *Bacteriological Analytical Manual* (BAM) Chapter 5. Overnight enrichments were tested in parallel with the LAMP screening assay and traditional culture methods. Aerobic plate counting and most probable number analyses were performed on all matrix sets. The relative level of detection (RLOD) was calculated following a paired study design.

Results: Out of 27 matrices, 25 were successfully validated as evidenced by fractional recovery at 25-75%, clean uninoculated samples, and acceptable RLOD values below 1.5. Two matrices (dust and pig ear) had false-positive results in the uninoculated test portions by BAM and/or LAMP due to cross-contamination events not related to method performance.

Significance: The matrix extension of this rapid, reliable, and robust *Salmonella* LAMP method to nine ISO categories of human food and animal food will have a far-reaching impact on protecting the safety of the nation's food supply by streamlining laboratory workflows towards better ensuring product safety.

P1-211 BPW and UHT Enrichment Protocols to Detect *Salmonella* in Chocolate Using the Assurance® GDS for *Salmonella* Tq Assay

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Introduction: *Salmonella* can persist in chocolate matrices. Due to complex matrices and the presence of inhibitory substances in chocolate, *Salmonella* can be difficult to detect. This is a significant concern to the food industry. Two new enrichment methods for Assurance® GDS for *Salmonella* Tq (GDS *Salmonella*) are presented for the detection of *Salmonella* in 375 g chocolate raw materials and finished products.

Purpose: To validate specific protocols to detect *Salmonella* in raw and finished chocolate products using the GDS *Salmonella* method.

Methods: Sensitivity and Relative Limit of Detection (RLOD) studies were conducted at external expert laboratory. Raw and finished chocolate products (375 g test portion) were inoculated by *Salmonella* strains and enriched for 24-32 h at 34-38°C using BPW (1:10 dilution ratio) or UHT milk containing 0.018 g/L of Brilliant Green (UHT+BG) dye (1:10 ratio). Enriched samples were diluted in wash buffer (100 µL/900 µL for BPW and 300 µL/700 µL for UHT+BG) before analysis. The GDS *Salmonella* method was performed, followed by ISO method confirmations. These protocols were compared to the ISO 6579-1 reference method for detection of *Salmonella*.

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Results: The sensitivity study and the RLOD values meet the Acceptability Limits (respectively 3 and 2.5) of an unpaired study design. For example, 21 samples were analyzed using UHT milk + BG protocols. 2 positive deviations and 3 negative deviations were obtained (ND-PD=-1<AL). Statistics support there is no significative difference between the GDS *Salmonella* method for both enrichment methods and the ISO reference method.

Significance: These data demonstrate allowance of two different enrichment methods for the detection of *Salmonella in* chocolate products by Assurance® GDS for *Salmonella* Tq. The GDS method detects *Salmonella* in 375 g chocolate raw materials and finished goods after a minimum enrichment time of 24 h in UHT+ BG or BPW broths.

P1-212 Polyethersulfone-Based Microfluidic Device Integrated with DNA Extraction on Paper and Recombinase Polymerase Amplification for the Detection of *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: The incidence of foodborne illnesses caused by *Salmonella* infections has been reported to increased significantly partially due to the industrialization of food production and complex global supply chain.

Purpose: The objective of this study was to develop a rapid and portable sample-to-answer microfluidic "lab-on-a-chip" device for the rapid detection of *S. enterica* in different types of food.

Methods: The microfluidic "lab-on-a-chip" device incorporates a cellulose paper strip for DNA extraction, two polyethersulfone (PES) membranes for nucleic acid amplification using recombinase polymerase amplification (RPA), and a lateral flow device for visualizing the amplicons. The specificity and sensitivity of the RPA were assessed for pure bacteria culture in tube and on PES-microfluidic device. The optimized PRA reaction and microfluidic devices were then applied to analyze *Salmonella* in different food products (*i.e.*, lettuce, chicken breast, and milk).

Results: The PES-based microfluidic device was confirmed to provide high sensitivity (*i.e.*, 260 CFU/mL) and 100% specificity to differentiate 7 *S. enterica* strains from 7 non-*Salmonella* strains. The overall sample-to-answer analysis can be completely within 30 min including DNA extraction, amplification and results readout. For analyzing real food samples, the PES-based microfluidic device successfully detected *Salmonella* in lettuce, chicken breast, and milk at concentrations of 6 CFU/g, 9 CFU/g, and 58 CFU/mL, respectively, with a 6-hour enrichment.

Significance: This sample-to-answer PES-based microfluidic device demonstrated excellent performance for rapid detection of *Salmonella* in food products and has great potential to be adapted by governmental laboratories and testing industries for routine analysis of pathogens in various food products.

P1-213 A Pipeline Approach to Identifying *Salmonella* Bacteriophages with Tail Spike Proteins

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Introduction: Bacteriophage (phage) tail spike proteins (TSPs) with lipopolysaccharide degrading activity have recently gained interest as antimicrobials for control of foodborne pathogens.

***Purpose*:** A semi-automated, high-throughput pipeline to identify *Salmonella* phages with TSPs was developed.

***Methods*:** Twenty-four *Salmonella enterica* isolates were used for phage isolation. Each host was individually added to wastewater samples and 10X tryptic soy broth followed by incubation at 37°C for 18 hours. Next, the samples were centrifuged (8,000 rpm, 10 minutes) and filtered through 0.22um filters. Using a liquid handling robot, the bacterial host, and 100uL of the corresponding filtrate was added to 96-well microtiter plates and incubated overnight at 37°C. Phage presence was determined by optical density measurements (OD600nm). Phages producing plaques with halos (indicative of a TSP) were isolated using the double overlay method and automated DNA extraction was accomplished, followed by a high-throughput PCR assay to indicate TSP presence using 13 primer sets representing the 16 different *Salmonella* phage TSP groups so far identified. Phages with TSPs were confirmed by transmission electron microscopy (TEM) and subjected to whole genome sequencing (WGS) using MiSeq paired-end sequencing.

***Results*:** The semi-automated procedure (from initial phage enrichment to the WGS step) was completed in 4.5 days. Thirty-five phages produced plaques with halos. The phages infected *Salmonella* isolates from 7 serotypes (Arizonae, Branderup, Enteritidis, Infantis, Kentucky, Reading, and Typhimurium). The PCR assay produced an amplicon indicating a potential TSP in six phages. TEM confirmed the presence of a TSP in 3 of the 6 phages, and sequence analysis indicated that the TSPs belonged to 2 of the 16 *Salmonella* TSP groups.

Significance: *Salmonella enterica* continues to cause foodborne outbreaks globally. This pipeline approach can be used to rapidly identify TSPs on *Salmonella* phages as a first step in development of a natural antimicrobial to control *Salmonella* in foods.

P1-214 Improvement of BAM *Salmonella* Culture Method for Sprout Using Large Test Portion Size

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Introduction: Sprouts has been associated with nine *Salmonella* outbreaks in US from 2012-2022. Using a larger test portion size of sprout than current 25 g analytical unit would increase the probability of detecting *Salmonella* that may be present in the production batch of sprouts.

Purpose: To determine an appropriate large sprout test portion size, sample to broth ratio and pre-enrichment incubation temperature to improve current FDA BAM *Salmonella* culture method for sprouts.

Methods: Alfalfa sprouts were inoculated with *Salmonella* Typhimurium at 0.79 CFU per test portion at four different test portion size such as 25g (reference method), 100g, 150g and 375g. Twenty replicates of each test portion size at 1:3 or 1:9 sample-to-broth ratio were pre-enriched in universal pre-enrichment broth and incubated at 35 ºC or 42 ºC. The BAM *Salmonella* culture method was followed thereafter. FDA qPCR, Gene up Salmonella, VIDAS Easy *Salmonella* and digital PCR as rapid screening methods were also used in the paired study for comparison.

Results: The pre-enrichment incubation temperature 42 ºC was more effective than current method incubation temperature 35 ºC for the detection of *Salmonella* in all test portion size of sprouts. There was no significant difference (p>0.05) between 25 g at 1:9 ratio and large test portion size (100g, 150g and 375g) at 1:9 ratio. However, the lower qPCR Ct values in 25g than in large test portion size were observed. The late qPCR Ct values from the large test portion size could be due to *Salmonella* population in pre-enrichment culture diluted by a large sample to broth ratio. VIDAS Easy generated significantly false negatives compared to Gene-up *Salmonella* assay. Digital PCR can be used as a second screening tool for the test portions with late Ct values by qPCR.

Significance: This study promotes more research for developing an effective test method for the detection of *Salmonella* in a large test portion size of sprouts.

P1-215 30-Minute-Screening of Microorganisms for Plant- and Milk-Based Dessert Products with the Hygiena Innovate™ System

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Introduction: The conversion of adenosine triphosphate (ATP) from metabolic processes of living cells to adenosine diphosphate can be measured using light detection systems. The Innovate™ system can detect microbial contamination using this rapid bioluminescence-based method. It is a sterility control option not only for liquid products but also for viscous dessert products.
Propose: The objective of this study was to show that the 30-minute Innovate-based sterility test for milk- and plant-based desserts produces qualitative results comparable to the plate count-based reference method after 72 hours incubation.
Method: Microbial cultures from six gram-positive and gram-negative bacteria, spore-formers and yeasts were prepared in corresponding enrichment broth. Defined inoculum concentrations between 1 and 20 CFU/sample were spiked directly into five different dessert matrices. Spiked and uninoculated product packs were incubated up to three days at 30 °C. After product incubation, aliquots of the product were taken for parallel testing using the Innovate System and plate-count methods. Tryptic soy agar and Sabouraud dextrose agar were used for plate-count testing. Innovate measurement was conducted using the RapiScreen™ Dairy Kit.
Results: All spiked organisms were detected by the Innovate RapiScreen Dairy system, if the organisms also grew on plates. After 72 hours of product incubation at 30 °C, the Innovate results corresponded 100% to the plate-count measurements (requiring a second plate incubation). The results were independent of plant- or milk-based production, extended-shelf-life (ESL) or ultra-high-temperature processing (UHT). This demonstrates that the Innovate system is suitable for commercial sterility testing of challenging dessert matrices, including chocolate cream products.
Significance: ATP detection with the Innovate RapiScreen Dairy system is a rapid 30-minute test for aseptically processed dessert products. Without a time-consuming second plate incubation, it generates comparable results to the reference method and shortens product release by more than 3 days.

P1-216 Screening Method for Thermophilic Spore-Formers in Plant-Based Drinks with the Hygiena® Innovate™ System

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Introduction: Rapid adenosine triphosphate (ATP) based detection of microorganisms can be used for sterility testing in a wide range of products. Spore-formers pose a challenge for ATP-based systems because there is no metabolic activity in spores; thus no ATP is measurable. Therefore, optimal time points for measurement between germination and resporulation must be determined. Different thermophilic spore-formers were investigated using the InnovateTM System, to demonstrate the possibility of detecting spore-formers with a bioluminescence-based ATP method.
Purpose: In this study, the detection of five different thermophilic spore-formers in three plant-based drinks using the Innovate RapiScreen™ Dairy system were demonstrated compared to plate count-based methods.
Method: Seven thermophilic strains, *Geobacillus, Anoxybacillus and Aneurinibacillus*, were spiked directly into the plant-based Oat-, Almond- and Soy-based drink products. Samples were spiked with a low-level of the organisms. After incubation for 24, 48 and 72 hours at 48 °C and 55 °C, samples were analyzed with the RapiScreen Dairy kit. In addition, the pH of the samples was measured. For results comparison, the ISO 4833-2:2013 method was conducted in parallel.
Results: The 48-hour product incubation at 48 °C was found to be optimal for the detection of the thermophilic organisms using the Innovate System, due to the highest ATP production after 48 hours. This resulted in high RLU values, demonstrating that the cells are in vegetative phase. One exception was *Anoxybacillus kamchatkensis* in Almond-Drink: After 24 hours (high RLU values), it sporulated, but pH value dropped significantly by 1.5 units in the vegetative phase. Therefore, the solution is a combined pH and ATP measurements that allow the detection of all spore-formers at one time point (48 hours).
Significance: The difficult detection of most common thermophilic spore-formers in plant-based drinks at one time point (48 hours) can be reached using the Innovate System when combined with pH measurement.

P1-217 Detection of *Listeria* in Yeast Samples with foodproof *Listeria* plus *L. monocytogenes* LyoKit

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Introduction: High background impairs the effective growth and detection of organisms by PCR. The detection of *Listeria sensu stricto* and *Listeria monocytogenes* in compressed and dry yeast products is challenging. A selective enrichment for *Listeria* and the inhibition of yeast growth is required. Therefore, different enrichment and DNA isolation options were tested in a paired study in comparison to the reference ISO 11290-1:2017-05 method.
Purpose: The study demonstrates the opportunity for detecting *Listeria sensu stricto* and *Listeria monocytogenes* in a high background flora of yeast products.
Method: Active dry and compressed yeast with a sample size of 25 g were inoculated with a low-level spiking of *Listeria monocytogenes.* Enrichments with a dilution in Actero (1:7), or tryptic soy broth (TSB, 1:20), with and without supplements, as well as subculturing options were tested after 24 and 48 hours at 37 °C. DNA isolation was conducted with two different Hygiena® methods. The alternative PCR method, foodproof® *Listeria* plus *L. monocytogenes* LyoKit, was used for analysis.
Results: A selective enrichment in Actero medium and TSB can generate reliable results for compressed yeast samples after 24 hours and for dried yeast after 48 hours. For the detection of *Listeria* in dried yeast, subculturing is required, where the PCR results were 100% concordant with the ISO method in which seven of eight replicates were positive for TSB enrichment (8/8 for Actero). For compressed yeast, 100% agreement with the reference method (8/8) was achieved after 24 hours enrichment time in both media without antimycotic supplements. The easy-to-handle DNA extraction methods BAX® Lysis and foodproof® StarPrep Two Kit, can be used with comparable sensitivity.
Significance: Concentrations of 1-10 CFU/25 g of *Listeria monocytogenes* could be successfully analyzed in 25 g samples of active dry and compressed yeast using the real-time PCR foodproof® *Listeria* plus *L. monocytogenes* LyoKit.

P1-218 Homogenizing the Detection Method for *Salmonella* in Liquid Whole Eggs with the Method for Shell Eggs

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Introduction: In the FDA's Bacteriological Analytical Manual (BAM), the detection of *Salmonella* from shell eggs (BAM C2.a) has been updated and could be completed in one week; while the protocol for liquid whole eggs (homogenized) (BAM C2.b) adds an extra 4 days (including weekend work).
Purpose: This study aims to harmonize the key steps of the protocols for the detection of *Salmonella* from shell eggs and liquid whole eggs and complete the detection of *Salmonella* from liquid whole eggs in one week.
Methods: An egg outbreak *Salmonella* Enteritidis strain (SAL02957) and a chicken *Salmonella* Typhimurium strain (SAL00723) were used to inoculate homogenized liquid whole eggs in four independent trials. Our modified method differed from the BAM protocol for detecting *Salmonella* from liquid whole eggs by eliminating the 96 h room temperature storage and incubating the entire 375 g eggs using eggs:pre-enrichment broth ratio of 1:2 instead of 1:9. Every trial comprised 60 test portions with 30 allocated to the BAM method and the other 30 to the modified method. With each set of 30 test portions, 20 are for fractional testing (inoculation level about 1 CFU/375g), 5 for positive controls (inoculation level about 10 CFU/375g), and 5 for negative controls.
Results: Data showed that the BAM method resulted in 50 positives among 80 test portions, while the modified method produced 55 positives among

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80 test portions, which was not significantly different statistically (p=0.5057). All the 40 positive controls yielded positive results, and all 40 negative controls were tested negative.

Significance: The data revealed that the modified simpler and shorter analytical protocol is as effective as the current BAM method for detecting *Salmonella* from liquid whole eggs.

P1-219 Recovery of *Salmonella* in Ground Yellow Mustard Seed – A Challenging Food Matrix with a Lethal Effect

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Introduction: Mustard seed inhibitory compounds are lethal to pathogens during analysis and thereby bias analytical results to non-detection if not properly treated. The lethal effect of compounds such as allyl-isothiocyanate create a "self-sanitizing" effect that must be countered to allow detection. The use of sample dilution was investigated as a means to prevent die-off of *Salmonella* during enrichment leading to a false negative result.

Purpose: To assess whether increasing enrichment dilution will reduce the level of toxic compounds in a sample promoting the detection of *Salmonella.*

Methods: An appropriate level of dilution was determined. 25g samples of ground yellow mustard seed (mustard) (1of 5 matrices) diluted 1:10, 1:20 or 1:50 in Trypticase-Soy broth or Buffered Peptone Water (n=18) were inoculated with 30CFU *Salmonella* Typhimurium, incubated 22h at 35˚C following the FDA-BAM detection method. All incubated enrichments were streaked to XLD and chromogenic agars and examined for typical colonies. A lethality study assessed the "self-sanitizing" effect. *Salmonella* was inoculated at a 4-log level into 25g mustard samples (n=24) diluted with BPW 1:10 or 1:50 (5-fold larger buffer volume), incubated at 35°C for 22h. Levels were assessed at time 0, 10 minutes, 60 minutes and 22h.

Results*: Salmonella* was not detected in any inoculated or uninoculated mustard samples diluted 1:10 or 1:20. Detection was possible in 1:50 enrichments (n=6). In the lethality study, a 2-factor ANOVA log CFU by time and dilution showed a significant difference between the two dilutions. The 1:10 dilution showed a 3.34 log reduction from T=0 (*Salmonella* not detected) at 22h while the 1:50 counts were stable from T=0 to 60 minutes (acclimation period) then increased by 5.48 logs at 22h.

Significance: Inhibitory compounds in the mustard matrix are lethal to *Salmonella* in standard 1:10 enrichment dilutions. However, with the appropriate dilution in the primary enrichment, *Salmonella* can be successfully recovered.

P1-220 Optimization and Evaluation of Modified Enrichment Broth for Rapid Detection of *Listeria monocytogenes*

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Introduction: A new enrichment broth is required for rapid detection of *Listeria monocytogenes*, a foodborne pathogen causing listeriosis, as current methods involving enrichment step are time-consuming.

Purpose: This study was conducted to develop modified enrichment broth *of L. monocytogenes* (MEB-LM) suitable for rapid detection of *L. monocytogenes* using the modified Gompertz model and response surface methodology (RSM).

Methods: In order to develop an MEB-LM enhancing buffered peptone water (BPW) with carbon, nitrogen, and mineral sources, this study selected eight carbon, six nitrogen, and five mineral sources as candidate components using Biocyc. To select components, we measured the growth curve of *L. monocytogenes* at 37 °C at 30 min intervals for 12 h by reading the absorbance at 600 nm with a microplate reader. The modified Gompertz equation modeled growth kinetics, and response surface methodology identified optimal component concentrations for maximum growth rates. For pH and temperature optimization, bacterial enumeration was compared at 0 h and 16 h under pH 5.8, pH 6.6, and 34, 37, and 40 °C. The efficacy of the MEB-LM was evaluated by comparing bacterial enumeration with BPW, Buffered Listeria Enrichment Broth (BLEB), and Fraser broth for 18 h at 3 h intervals.

Results: D-(+)-mannose, L-threonine, and magnesium sulfate were identified as additional key components for MEB-LM through the comparison of growth curves. The optimum concentrations of D-(+)-mannose, L-threonine, and magnesium sulfate determined through the modified Gompertz equation and response surface methodology were 19.2 g/L, 7.9 g/L, and 17.9 g/L, respectively. MEB-LM showed maximum growth-promoting effects under pH 6.6 and 34℃ conditions. After comparing the bacterial growth after 18 h, MEB-LM showed the highest level at 8.741 ± 0.164 Log CFU/mL compared to the conventional enrichment broths.

Significance: The MEB-LM will contribute to enhancing food safety by concentrating *L. monocytogenes* more rapidly than conventional enrichment broth.

P1-221 Evaluation of *Listeria* Special Broth II (LSB II) for Recovery and Detection of Non-Stressed and Heat Stressed *Listeria* spp.

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Introduction: LSB II is Bio-Rad Laboratories' latest second-generation enrichment media that allows for detection of *Listeria* spp. at 37°C at 18-24 hours post-incubation on the iQ-Check PCR platform. NQAC Dublin is currently evaluating this medium for potential implementation.

Purpose: The objective of this study was to assess the performance of LSB II for detection of healthy and heat-stressed *L. monocytogenes* and *L. innocua* at minimum and maximum incubation times (18 and 24 hours) in validated and non-validated enrichment volumes (225ml and 1125ml, respectively).

Methods: Healthy and heat-stressed cultures (treated at 56°C for 15 minutes) were inoculated into 225 or 1125ml of prewarmed LSB II and incubated at 37°C for 18 and 24 hours. At both time points, aliquots were taken for enumeration onto RAPID'L.mono agar, and for PCR analysis on the iQ-Check *Listeria* spp. and *L. monocytogenes* II kits. Experiments were replicated three times on three different days, and statistical analyses were performed using Minitab's General Linear Model function.

Results: Both healthy cultures of *L. monocytogenes* and *L. innocua* grew to 6.81 and 6.89 log CFU/ml after 18hrs incubation, respectively, and greater than 9 log after 24hrs incubation in 225ml enrichments. In the non-validated 1125ml enrichment volume, concentrations of healthy cultures were around 1 log lower than the 225ml volume. Heat-stressed *L. monocytogenes* and *L. innocua* grew to around 3-4 log CFU/ml in both enrichment volumes after 18hrs incubation, and 6-7 logs after 24hrs. Over three replicates, *L. monocytogenes* and *L. innocua* were successfully detected as soon as 18 hrs of incubation. The effects of culture type, stress, enrichment volume, and incubation time were statistically significant (p<0.05).

Significance: LSB II demonstrated great potential for rapid detection of *Listeria* in routine analyses, but further work needs to be conducted. Future studies will evaluate the performance of LSB II with Nestle food matrices and environmental samples.

P1-222 Evaluation of a Carbon Dioxide Sensor in a Closed System with *Listeria* spp. Specific Broth

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Introduction: Carbon dioxide sensor in a closed vial may be used to detect pathogen growth from environmental surface and food samples in real time. *Listeria* spp. selective broth may be used in a closed CO2 system to enhance detection time.

Purpose*:* This work evaluated CO_2 detection of *Listeria innocua* and *Listeria monocytogenes* from dried surfaces containing food residues in comparison to Chromogenic Listeria Agar.

Methods*:* CertaBlue (CB-MCS Diagnostics) sensor vials were adapted with a swab-attached cap (Charm Sciences, Inc.) and 9 mL PDX-LIB media (Para-

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digm Dx, Inc.) were added into the vials. Serial logarithmic dilutions of overnight cultures, *Listeria innocua* 33090 and *monocytogenes* 19118 in TSB, were diluted in TSB. A one-milliliter volume of log-1 (high), log-2 (medium), and log-3 (low) dilutions were added to 1:10 food dilutions of ice cream and chicken nuggets, then 100 µL inoculated onto 100 cm^2 stainless-steel coupons in duplicate. Inoculations were spread with pipet tip and left to dry >0.5 hour at room temperature. PUR-Blue™ Swabs with 1mL HiCap broth (World BioProducts, Chicago) were used in duplicate to swab the stainless-steel coupons. These swabs were split, and one added to the CB vials with adapted cap and incubated for 48 hr at 37°C in the CertaBlue AutoScanner. The second swab was extracted into HiCap broth and plated on Chromogenic Listeria Agar (CLS) plates for confirmation and enumeration of *Listeria innocua* and *monocytogenes*.

Results: Presumptive results at low levels <10 CFU/swab were detected on CB within 30 hours and confirmed at same *p*=0.33 as CLS. Higher levels >100-1000 CFU/Swab were detected in 16-22 hr on CB and confirmed 100% positive n=6 with CLS. Drying on surfaces caused 2 to 3 decimal reductions in cell population and was not influenced by food residue. No food residue interference was observed.

Significance: *Listeria* Indicator Broth and CertaBlue technologies can rapidly identify presumptively *Listeria* positive swab samples.

P1-223 Evaluation of a Chromogenic Plating Medium for the Isolation of *Bacillus cereus* Group

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Introduction: *Bacillus cytotoxicus*, being the most genetically diverse species within the *Bacillus cereus* group and recognized as a virulent member, presents challenges in the detection and isolation of these foodborne pathogens.

Purpose: The study aimed to assess a chromogenic plating medium designed for the isolation of *B. cytotoxicus* and other members belonging to the *Bacillus cereus* group, by targeting the presence of phosphatidylcholine-phospholipase C activity.

Methods: Inclusivity and exclusivity evaluations were performed using a total of 30 *Bacillus cereus* group and 47 non-target bacterial strains. Bacterial strains were grown in brain heart infusion broth and streaked onto prepared plates of R & F® *Bacillus cereus* Group Chromogenic Plating Medium and Tryptic Soy Agar (TSA). After incubation at 35°C for 24 hours, microbial growth and bacterial colony morphology were compared between both media. Moreover, plating efficiency of the chromogenic agar was determined using the spread-plate technique and compared against TSA prepared plates.

Results: The inclusivity and exclusivity of the chromogenic agar plating medium was 100% and >95%, respectively. In addition, the plating efficiency of select strain was greater than 90%. The colony morphology of species within the *Bacillus cereus* group, including *B. anthracis*, B. *cereus sensu stricto*, *B. cytotoxicus*, *B. mycoides*, *B. thuringiensis*, *B. toyonensis*, *B. wiedmannii*, and *B. weihenstephanensis*, exhibited a dark to medium turquoise color, ranging from 1 to 3 mm in diameter, with or without a cream ring. In the case of *B. pseudomycoides*, colonies presented as turquoise rhizoid-shaped, while other *B. cereus* group species displayed circular smooth colonies, with either entire or irregular edges.

Significance: The chromogenic plating medium could improve the effectiveness of detection and isolation methods for all members of the *Bacillus cereus* group, as defined by the FDA's Bacteriological Analytical Methods, including the newly recognized *B. cytotoxicus*.

P1-224 Assessment of the Romer Labs® RapidChek® *Campylobacter* Test Using Four *Campylobacter*-Specific Enrichment Broths

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Introduction: Rapid and accurate detection of *Campylobacter jejuni* and *Campylobacter coli* is crucial due to their prevalence in poultry.

Purpose: The objective of this study was to verify the limit of detection, inclusivity, and exclusivity of an antibody lateral flow device when detecting *Campylobacter* using four separate enrichment broths.

Methods: C. jejuni NCTC 11351 was inoculated into test tubes containing either 10 ml of Bolton broth (2 x concentrations), Hunt's Broth, R & F® *Campylobacter* Enrichment Broth, or Romer® *Campylobacter* Broth. Each tube was incubated at 42°C for 48 hours under microaerophilic conditions, serially diluted by a factor of 10 to obtain seven tubes per broth containing different bacterial detection levels ranging from 10^1 to 10^7 CFU/ml. The Romer Labs® RapidChek® *Campylobacter* Test was performed for each inoculated broth at each concentration level, and results were recorded 10 minutes after application. Statistical analysis was performed using the chi-square test to compare the results obtained with each broth. Additionally, select strains of *Campylobacter* (n=3) and *Arcobacter* (n=18) were grown in Bolton broth (1 x concentration) at 42°C for 48 h under microaerophilic conditions and aerobically at 30°C for 48 h, respectively. After incubation, the antibody lateral flow device was tested for each culture, and percent inclusivity and exclusivity were calculated.

Results: The *Campylobacter*-specific antibody lateral flow device achieved a limit of detection of greater than or equal to 10^5 CFU/ml for each inoculated enrichment broth sample. No significant difference ($p > 0.05$) was observed when among the four enrichment broths when applying the antibody lateral flow device. Moreover, the screening method achieved 100% inclusivity and 100% exclusivity when testing against select strains of *Campylobacter* and *Arcobacter*.

Significance: This study demonstrates that the antibody lateral flow device is a robust and sensitive screening method that can be effectively incorporated for *Campylobacter* testing in foods.

P1-225 Influence of *Campylobacter* Enrichment Conditions on Isolation and Characterization Outcomes for Environmental Poultry Samples

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Introduction: *Campylobacter*, a common cause of foodborne illness attributed to poultry, is an organism that is difficult to culture, particularly from samples containing high and diverse microbial populations. Methodological limitations create challenges in characterization of *Campylobacter* dynamics in the poultry production chain.

Purpose: To evaluate effects of enrichment media on isolation and quantification of *Campylobacter* spp. in pre-harvest poultry matrices.

Methods: Environmental samples comprising bootsock, drag, nest box, nest box slat, egg belt, egg room floor swabs, and litter were collected from poultry breeder houses (n=4) in the Southeastern U.S. on two visits (n=2), one month apart. Composite samples of each type per poultry house were stomached in buffered peptone water (BPW), mixed 1:10 with Bolton or Exeter broth containing 5% lysed horse blood and supplemented with antibiotics, and incubated at 42°C under microaerophilic conditions for 24 h. *Campylobacter* spp. prevalence was determined using real-time PCR and confirmation of presumptive colonies following streaking onto Campy-Cefex and Campy-Line agar.

Results: *Campylobacter* spp. was isolated more frequently from Exeter broth than Bolton broth, particularly for bootsock (2/8 samples positive, Bolton; 6/8, Exeter), drag swab (1/8, Bolton; 5/8, Exeter), and litter (1/8, Bolton; 8/8, Exeter) ($p < 0.05$). Bolton broth preferentially enriched for *C. coli* (78% of positive samples, Bolton; 50% of positive samples, Exeter). Bolton broth with plating on Campy-Cefex failed to isolate *Campylobacter* for all PCR-confirmed positive samples (n=38). Conversely, *Campylobacter* was isolated on Campy-Line for 100% of PCR-confirmed positive samples, regardless of enrichment broth. All PCR-positive litter samples (n=8) contained *C. coli* and *jejuni*. The relative concentrations of *C. jejuni* were relatively increased in Exeter compared to Bolton, as assessed by Ct values of the paired samples ($p < 0.05$).

Significance: The choice of enrichment media can impact the likelihood of isolating *C. coli* and *jejuni* from poultry environmental samples.

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P1-226 Methodology for Improved *Campylobacter* Isolation and Detection in Food Products

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Introduction: The protocol presents a rapid yet robust method for isolating *Campylobacter* spp. from food sources.

Purpose: The objective is to provide improved methodologies for the isolation and identification of *Campylobacter* spp. from food sources in a manner that is aligned with the criteria and constraints of established standards used by regulatory agencies like the Food and Drug Administration and the U.S. Department of Agriculture.

Methods: Central to this protocol is the collection of rinsates from various meat samples, followed by centrifugation and resuspension in specialized growth media. The samples undergo a 24-hour incubation period at 42°C under controlled microaerobic conditions to facilitate *Campylobacter* growth. Utilizing the spiral-shaped morphology and corkscrew motility of *Campylobacter* spp., the protocol integrates a passive filtration technique to isolate these cells. This filtration process leverages the highly motile nature of *Campylobacter*, allowing cells to traverse membrane filters, resulting in selective isolation of *Campylobacter*. For species-level identification, the methodology integrates a multiplex real-time polymerase chain reaction assay, ensuring precise identification and characterization of the isolates.

Results: The optimized protocol successfully isolated 49 pure *Campylobacter* strains (36 *C. jejuni*, 13 *C. coli*) from a selection of 79 meat samples, demonstrating its efficacy in recovering and identifying these pathogenic species. The growth medium employed notably enhanced the recovery of Campylobacter cells, reducing the required enrichment duration by 50%. Comparative analysis of filtration membranes demonstrated 0.65 µm pore-size filter significantly outperformed the 0.45 µm pore-size filter (p<0.0001), yielding an average 29-fold higher recovery of cells from the enrichment. Furthermore, a high moisture content in the agar plate can hinder filtration.

Significance: This protocol aligns with established sampling standards, while addressing limitations within current methodologies. Its application holds promise for enhancing public health by contributing to epidemiological studies and potentially reducing *Campylobacter*-related infections associated with poultry consumption.

P1-227 Independent Laboratory Study for the Validation of the RAPID'*Campylobacter* Chromogenic Agar Method

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Introduction: RAPID'*Campylobacter* Agar is a selective chromogenic agar for the detection of thermophilic *Campylobacter* species in select foods and environmental samples.

Purpose: The purpose of this AOAC Independent Laboratory Study was to compare the candidate method using traditional and alternative plating methods to the USDA/FSIS MLG 41.07 before and after enrichment with Hunt Broth for 22-26 hours at 42 ± 1 °C under microaerobic conditions.

Methods: The candidate method traditional and alternative plating methods were compared to the U.S. Department of Agriculture Food Safety and Inspection Service *Microbiology Laboratory Guidebook (MLG) 41.07 Isolation and Identification of Campylobacter jejuni/coli/lari f*rom Poultry Rinse, Sponge, and Raw Product Samples following a paired study design for the matrix study. The candidate methods were further evaluated in an inclusivity and exclusivity study where 50 *Campylobacter* isolates and 30 non-*Campylobacter* isolates were evaluated. In addition, a product consistency and stability study evaluated three separate lots of dehydrated and ready-to-use agar, and a robustness study evaluated dehydrated and ready-to-use agar evaluating critical parameters.

Results: In the inclusivity and exclusivity study, 50/50 *Campylobacter* isolates were detected, and all 30/30 exclusivity organisms were not detected. Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. The candidate method demonstrated no statistically significant differences between candidate and reference methods results (dPOD=0) for raw ground chicken (POD 0.45), chicken carcass rinse (POD 0.60) and turkey carcass sponge (POD 0.35), except for the traditional plating method before enrichment (POD -0.30. -060, -0.15, respectively). The product consistency and stability showed there was no significant impact on the performance of the agar over time. The robustness evaluation demonstrated that minor changes to operational parameters have no significant impact on the performance of the agar.

Significance: The use of this novel chromogenic agar allows for a fast and complete workflow for the detection of *Campylobacte*r spp.

P1-228 Independent Laboratory Study of a Real-Time PCR Assay for the Detection of *Campylobacter* in Poultry Products

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Introduction: The iQ-Check *Campylobacter* kit employs real-time PCR technology for the accurate detection of thermophilic *Campylobacter* species from raw ground chicken (325 g), chicken carcass rinse (30 mL), and turkey carcass sponge (sponge) after enrichment in Hunt Broth for 22-26 hours at 42 ± 1 °C under microaerobic conditions.

Purpose: The purpose of this AOAC Independent Laboratory Study was to compare the candidate method to the USDA/FSIS MLG 41.07 for raw ground chicken, chicken carcass rinse, and turkey carcass sponge for a Level 3 Method Modification for enrichment in Hunt Broth for 22-26 hours at 42 ± 1 °C under microaerobic conditions.

Methods: The rapid method was compared to the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook (MLG) 41.07 Isolation and Identification of *Campylobacter jejuni/coli/lari f*rom Poultry Rinse, Sponge, and Raw Product Samples following a paired study design for all matrices. The rapid method was further evaluated in an inclusivity and exclusivity study where 50 *Campylobacter* isolates and 30 non-*Campylobacter* isolates were evaluated.

Results: In the inclusivity and exclusivity study, all 50/50 *Campylobacter* isolates were detected, and all 30/30 exclusivity organisms were not detected. Statistical analysis was conducted according to the Probability of Detection (POD) statistical model. The candidate method demonstrated no statistically significant differences between candidate and reference methods results (dPOD=0) for raw ground chicken (POD 0.45), chicken carcass rinse (POD 0.60) and turkey carcass sponge (POD 0.35). The product consistency and stability showed there was no significant impact on the performance of the agar over time. The robustness evaluation demonstrated that minor changes to operational parameters have no significant impact on the performance of the agar.

Significance: This rapid, PCR method allows for fast and reliable detection of thermophilic *Campylobacter* spp.

P1-229 Survival of Viable but Non-Culturable State *Campylobacter jejuni* by Co-Culturing with Human Small Intestinal Epithelial-Like Cells

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✦ ***Undergraduate Student Award Entrant***

Introduction: Foodborne illness caused by *Campylobacter jejuni* may be related to existence of viable but non-culturable (VBNC) state of the cells. Although it has been known that *C. jejuni* easily changes to VBNC state induced by some environmental stresses, infectivity of the *C. jejuni* in the VBNC state is still unclear.

Purpose: The aim of the present study was to investigate whether *C. jejuni* in VBNC state enables to infect (invade) human intestinal cells by co-culturing

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of *C. jejuni* in VBNC state with monolayer Caco-2 cells.

Methods: A cocktail of 11 strains of *C. jejuni* cultured with Bolton broth (10^8 CFU/mL) were air-dried for 12 h. The air-dried *C. jejuni* cells were resuspended in 250 µL of Dulbecco's modified Eagle's medium with supplements and co-cultured with Caco-2 monolayer cells in a micro-aerobic condition at 37 °C for up to 48 h. Enumeration of culturable *C. jejuni* cells by mCCDA medium were examined for extracellular and intracellular (invasive) of Caco-2 cells. Furthermore, LIVE/DEAD *Bac*Light assay and PMA-qPCR procedure were used to evaluate the VBNC state *C. jejuni*.

Results: Although culturable *C. jejuni* cells were completely reduced by an undetectable level (1 CFU/mL) after 12 h air drying, viable cells were observed by the LIVE/DEAD *Bac*Light assay and also quantified by PMA-qPCR method. While no culturable *C. jejuni* cells were detected in both the extracellular and intracellular of Caco-2 cells during co-culturing period, viable cells were still detectable extracellularly of the Caco-2 cell after 48 h in the LIVE/DEAD *Bac*Light assay.

Significance: VBNC state *C. jejuni* cells induced by air drying continued to survive during co-culture with Caco-2 cells for up to 48 h incubation. However, it was suggested that VBNC state *C. jejuni* cells may not invade into Caco-2 cells in a state of restored culturability.

P1-230 Evaluation of an Alternative Method for Enumeration of Microorganisms for Confectionary Samples from Argentina

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Introduction: The enumeration of indicator microorganisms in raw materials and finished products is commonly applied by the food industry for quality and regulatory compliance purposes. The effectiveness of applying a rapid methodology to detect the presence of these microorganisms is fundamental for taking corrective actions.

Purpose: The objective of the study is to determine that the performance of the alternative method intended enumeration of mesophilic aerobic microorganisms, total coliform bacteria, Enterobacteriaceae, and yeasts and molds, are suitable for their intended use, in the different matrices evaluated.

Methods: Four Petrifilm® Plates were used in this study. Twenty-three matrices were evaluated, and two microorganisms were individually spiked (*Enterobacter aerogenes* and *Saccharomyces cerevisiae*). Samples were diluted 1:10, dilutions were plated and incubated following protocols. One mL sample was plated onto each Plate Count Agar (ISO 4833), Crystal Violet-Red Neutral-Bile-Glucose Agar (ISO 21527) and Crystal Violet-Red Neutral-Bile-Lactose Agar (ISO 4832) with temperatures and incubation times according to the cited regulations. Likewise, one mL samples were grown in AC Plate, EB Plate and EC Plate and incubated at 35±1°C for 48 h (AC and EC) or for 24 h (EB). For yeast and molds, one mL sample was plated onto a plate of DG18 agar (ISO 4833) as well as on RYM Plate and incubated at 25±1°C for 48 h. After incubation, plates were enumerated manually and with an automated reader. A paired t-test was conducted to determinate statistical differences between interpretations (p<0.05).

Results: There was no statistical difference between the rapid and traditional methods tested. The use of the automated reader with the sample-ready medium showed comparable results with reference method, overall enabling productivity gains by saving media preparation time and decreasing interpretation issues.

Significance: The alternative method for microbial enumeration enabled reliable and rapid enumeration and offers an alternative rapid method for confectionary samples with accurate and repeatable results compared to the traditional agar.

P1-231 Evaluation of PetricoreTM Dry Film Media for the Enumeration of Total Aerobic Bacteria

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Introduction: Petricore™ AC (total aerobic count), a novel ready-to-use and dry rehydratable film media has been developed by PNG Biomed co., Ltd. for determining total aerobic colony counts. The aerobic plate count (APC) is intended to indicate the level of microorganisms in the products and commonly used in various industries such as food, dairy, and pharmaceuticals.

Purpose: The goal of this research is to compare the performance and applicability of Petricore™ with PCA method, the standard aerobic count method.

Methods: A paired validation study was carried out to compare Petricore™ with U.S. Reference methods (FDA BAM Chapter 3) for 13 different food matrices (2 meats, 3 fishes, 3 vegetables, 5 processed foods). In each matrix, five replicates at three contamination levels of samples were used. In addition, media performance test for 5 reference bacteria was conducted according to ISO 11133 guidelines. The colony count obtained by both methods were logarithmic transformed. Productivity ratio, mean difference, standard deviation of repeatability (S_r), relative standard deviation (RSD), and linear correlation coefficient (R^2) were determined. Statistical analysis was carried out with one-way ANOVA (p>0.05).

Results: Two methods showed high correlations of > 0.90 and yielded a mean difference within the acceptable range of ± 0.50 in all 13 food products. No significant differences (p>0.05) were observed for enumerating the aerobic bacteria. The standard deviation of repeatability of Petricore™ was 0.01-0.13, which is comparable to PCA with values of 0.02-0.12. Petricore™ demonstrated the precision of relative standard deviation (RSD) ≤ 3% for all food matrices. For the media performance test, Petricore™ obtained a productivity ratio ≥ 0.70 for five reference bacteria.

Significance: This study demonstrates that Petricore™ can be used as a reliable alternative method to the conventional plate method for the microbiological analysis in various food matrices.

P1-232 Advancing Quality Assurance in Cell-Culture Production Using the BacT/ALERT® System

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Introduction: In the burgeoning field of cell-culture alternative protein production, ensuring the sterility of media used in the cultivation process is paramount. Cultivation is performed in closed system bioreactors, meaning if contamination is present it will not be detected until the final product is ready, resulting in the loss of the entire batch and a high economic burden for the manufacturer as the cultivation process is currently expensive to perform. Rapid determination of culture media sterility is critical to making decisions on batch viability. The BacT/ALERT® 3D microbial detection system is a rapid method that uses specific bottles to detect the growth of aerobic or anaerobic microorganisms for the cell-culture industry.

Purpose*:* The objective of the study was to verify the alternative method is fit-for-purpose in detecting the target organisms in the proprietary growth medium.

Methods: A proprietary cell-culture growth medium was evaluated using both the BacT/ALERT iAST (aerobic) and iNST (anaerobic) culture bottles. Fourteen organisms were evaluated using the iAST bottles and 4 organisms were tested using the iNST bottles. Each organism was tested in triplicate. Testing was performed at 32°C until detection (< 7 days). Test portions were confirmed by plating an aliquot of each bottle onto non-selective agar to confirm the presence or absence of growth.

Results*:* Results of the study indicate that the alternative method rapidly detects low concentrations of the test organisms. The average detection time for bacteria was <1.7 days with one exception, *Cutibacterium acnes,* which required an average of 5.42 days. For two fungal organisms, *A. brasiliensis* and *R. glutinis,* the average rate of detection was 2.27 and 3.10 days, respectively. All controls evaluated in the study met the acceptance criteria.

Significance: The cell-culture industry can obtain sterility results an average of 4-5 days quicker for most organisms using the alternative method when compared to traditional culture methods.

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P1-233 Verification Study of Dietary Supplement Gummies using the GENE-UP® NutraPLEX™ PRO Method

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Introduction: The increased demand for vitamins, dietary supplements, and functional foods continues to expand and has resulted in overlapping categories. One example is gummies, or chewable gels as the designated by the USP, which were originally designed as candy for children but have become an extremely popular nutritional supplement dosage form for consumers. Gummies come in many forms, originally using gelatin as a base, with later versions incorporating pectin or modified starches, all while being combined with hundreds of different active ingredients. Ensuring these products are safe for consumers is not easy, as their composition can pose challenges to analytical test methods. The GENE-UP® NutraPLEX™ PRO assay is a real-time PCR assay for the simultaneous presumptive detection of *E. coli, Salmonella,* and *Staph aureus* from nutraceutical products.

Purpose: A method verification study was performed for the simultaneous detection of the three target analytes in four types of gummy dietary supplements.

Methods: Four dietary supplement gummies were evaluated each consisting of 7 inoculated replicates and 1 negative control of 25 g matrices. Each matrix was tri-inoculated with *E. coli, Salmonella* and *S. aureus* at ~5 CFU and enriched in a NutraPLEX universal enrichment broth at a 1:20 dilution. Products were screened by the PCR method and confirmed by directly streaking onto selective agars as well as following confirmation in USP <2022>.

Results: Results indicated positive detection for all three pathogen targets for all matrices screened and 100% alignment with both direct streaking and USP confirmations. All uninoculated controls tested negative for target assays and reference confirmations.

Significance: The data from this study supports the product claim that the alternative method can simultaneously detect *E. coli, Salmonella* and *S. aureus* in dietary supplement gummy matrices.

P1-234 Verification of TEMPO AC as Alternative Method for the Enumeration of Mesophilic Aerobic Bacteria in Raw Meat at a JBS Microbiology Laboratory

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Introduction: The enumeration of aerobic mesophilic bacteria is one of the most common analysis for quality indicator in the animal protein industry. Samples at different processing stages have a wide range of bacteria concentration, so an alternative and reliable rapid method that is capable of enumerating microorganisms in a large order of magnitude is desired.

Purpose: Verify the performance of the alternative method in accordance with the Brazilian method verification guideline DOQ/CGCRE-0089 (INMETRO) in comparison with AOAC RI approved protocol (No. 121204) using raw meat as a reference matrix.

Methods: Ten samples of *in natura* meat were sterilized prior to the experiment and were artificially contaminated with *Escherichia coli*. The spiking concentration was calculated to be 3 $\log_{10}$ CFU/g. Two operators performed the automated method workflow with the same ten samples, and their results were statistically compared in terms of repeatability (SD), reproducibility (SD_r), and measurement uncertainty (MU).

Results: The candidate method was statistically complaint with ISO 16140-3 and JBS internal guideline criteria in the three parameters evaluated (N=10; SD=3.99%; SD_r=0.115; MU=0.214).

Significance: TEMPO® AC is a validated and automated enumeration method that delivers reliable results in 24 hours, and each analysis has a 3.7log quantification range, allowing a streamlined workflow. Results are read, calculated, and interpreted by the system, minimizing the probability of human errors. The presented results have shown that, even with a limited representation of *E. coli* for the whole aerobic mesophilic group, the obtained results results are in accordance to the validation data and corroborate TEMPO® AC as an alternative for mesophilic aerobic microrganisms enumeration in animal protein samples.

P1-235 GENE-UP® SE/ST as a Suitable Method for Quick *Salmonella* Enteritidis and Typhimurium Detection by a Microbiology Corporate Lab in Poultry and Swine Products and Derivates

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Introduction: *Salmonella* Enteritidis (SE) and Typhimurium (ST) represent a concern for Public Health Systems, due to their relative prevalence in food and their growing antimicrobial resistance. Traditional mean of characterization in food relies on colony isolation followed by Kauffman-White serotyping.

Purpose: Assess a multiplex PCR method with endpoint analysis for streamlined detection of SE/ST in terms of false positive and negative rates, specificity, sensitivity and precision, as per Brazilian guidelines (Inmetro).

Methods: 30 samples previously analyzed and proven to be negative for *Salmonella spp.* were used for this study. 10 of them were artificially contaminated with 3-5 CFU of SE and/or ST suspensions and 500 CFU of other Enterobacteriaceae. 10 of them were artificially contaminated with *Salmonella* Heidelberg and the other 10 ones were blank samples containing *E. coli*.

Samples have undergone analysis by AOAC OMA 2020.02 while lysates were kept refrigerated. *Salmonella spp.* positive samples had their lysates analyzed by the candidate method while traditional confirmation of *Salmonella spp.* has been performed in parallel.

Results: GENE-UP® SLM 2 (AOAC 2020.02) was able to correctly detect *Salmonella spp.* in 20 artificially contaminated samples and correctly not detect *Salmonella spp.* in blank samples. The 20 lysates left were analyzed by GENE-UP® SE/ST: for the *Salmonella* Heidelberg samples group, the method has not detected SE or ST. For the 10 remaining samples, the alternative method was able to correctly detect SE, ST, or both. The traditional method confirmations were in accordance with the rapid method.

Significance: The alternative method has proven to be a quicker and simpler alternative to the laborious and complex Kauffmann-White scheme for *Salmonella* serotyping. It requires fewer training and experience when compared to the traditional serotyping and represents a feasible alternative for accurate SE/ST detection.

P1-236 Verification of GENE-UP® EH1 2 and GENE-UP® ECO 2 as Alternative Methods for Detection of the *stx* & *eae* Genes and *E. coli* O157:H7 in Raw Meat at a JBS Microbiology Laboratory

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Introduction: Animal protein market represents a significant portion the Brazilian agricultural sector. Shiga-toxin producing *Escherichia coli* (STEC) are often associated with foodborne outbreaks related to meat consumption. Rapid and reliable tests to detect this group are necessary to optimize laboratory turnaround time and to technically support decision-making process.

Purpose: Perform verification of a harmonized multiplex PCR method for the detection *stx* & *eae* genes and *E. coli* O157:H7 in a JBS Microbiology Lab.

Methods: An alternative method verification study was carried out using Brazilian government guidance document DOC-CGCRE-089 (by Instituto Nacional de Metrologia, Qualidade e Tecnologia) and aligned to JBS Friboi protocol. 20 raw meat samples known negative for the analytes of interest were used in the experiment. All samples were artificially contaminated with 100 CFU of the non-target *Staphylococcus aureus*, and ten were inoculated with 1-5 CFU

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of *E. coli* O157:H7. After artificial inoculation, samples were tested in accordance with AOAC RI Approved Protocol (Certificate No. 012805).

Results: Both GENE-UP® EH1 2 (*stx* & *eae*) e GENE-UP® ECO 2 (*E. coli* O157:H7) were successfully verified and methods were able to correctly detect the analytes in all ten artificially contaminated samples. Considering blank satisfactory results, Sensitivity, Specificity and Precision were 100%; False positive and False negative rates were 0%. Method was considered fit for purpose.

Significance: GENE-UP® EH1 2 and GENE-UP® ECO 2 are alternative validated methods that streamline and harmonize the laboratory workflow. From one enriched sample, it is possible to simultaneously screen the presence of *stx* & *eae* genes and, in case of detection, the same already-prepared lysate can be used to assess the presumptive presence of *E. coli* O157:H7.

P1-237 TEMPO® CAM at a Poultry Facility Factory Lab: An Automated Alternative for Thermotolerant *Campylobacter* Enumeration in Poultry Samples

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Introduction: Because it is very sensitive to oxygen exposure, the *Campylobacter* traditional method for enumeration can be tricky, and the microorganism might not be recovered as expected, especially for confirmation purposes. Laboratories and industries must not take the risk of being mistaken, as the relevance of *Campylobacter* for Global Public Health raises.

Purpose: Assess the performance of TEMPO® CAM using Brazilian guideline DOQ/CGCRE-0089 (INMETRO) for method verification in comparison with AFNOR validation 12/43 – 04/20.

Methods: 10 chicken carcass samples were rinsed with 400mL of buffered peptone water (BPW). Rinsate was recovered and had 1mL analyzed by TEMPO® CAM method by 2 operators. After enumeration at the equipment level, results were expressed as total number of cells in 400mL. In parallel, 10 raw chicken samples of 10g were artificially contaminated with *Campylobacter jejuni* in three concentration levels: low (10-99CFU/g), medium (100-999CFU/g) and high (1000-9999CFU/g). Results were expressed as CFU/g. Each sample has been analyzed by 2 operators and final results allowed the calculation of repeatability (SD) & reproducibility deviation (SD_r) and, finally, uncertainty measurement (MU), by each sample group.

Results: For the rinsate samples, SD=5.39%; SD_r=0.189 and MU=0.355. For the raw chicken samples, SD=5.65%; SD_r = 0.111 and MU=0.208. In all scenarios, the comparison of results complies with ISO 16140-3 AFNOR Validation data and the adopted guideline criteria, giving evidence that the method is fit for purpose.

Significance: TEMPO® CAM is the automation of a complex and meticulous method, the traditional method for *Campylobacter* (ISO 10272). In this context, TEMPO® has shown to be a good alternative for accurate results, with the advantage of being protected against common errors during *Campylobacter* analysis.

P1-238 MicroVal and NordVal Certification of Ready-to-Use Culture Media, Easy Plate CC for Enumeration of Coliforms in a Broad Range of Foods

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Introduction: Ready to use (RTU) media offers many advantages to the end user including less preparation and ease of use as well as being more sustainable. Easy Plate CC (Kikkoman Biochemifa Company) is a RTU device to enumerate Coliforms with greater selectivity compared to the traditional VRBLA.

Purpose: To compare the performance of the RTU device and ISO 4832:2006 for the enumeration of Coliforms in a broad range of foods following the ISO 16140-2 validation protocol.

Methods: This study evaluated the specificity, selectivity, repeatability, accuracy and relative trueness of the RTU device as required by ISO 16140-2. During the study, 225 food samples were analyzed across five food categories; dairy products, fishery products, produce and fruits, meat and poultry and multicomponent foods. All samples were tested according to the manufacturer's instructions and ISO 4832:2006.

Results: Results from the study revealed good agreement between the RTU device and the reference method in the 75 samples analyzed in the relative trueness study(Correlation coefficiet is 0.978). In the accuracy profile study, all 5 categories tested satisfied the 0.5 log acceptability limit or the recalculated acceptability limits. Data also indicated that the RTU device was selective and specific, differentiating more non-target organisms than VRBLA.

Significance: The RTU device gives equivalent results to the ISO reference method ISO 4832:2006 for a broad range of foods. This RTU device is a convenient alternative for the enumeration of coliforms.

P1-239 MicroVal and NordVal Certification of Ready-to-Use Culture Media Easy Plate EC for Enumeration of *E. coli* and Coliforms in a Broad Range of Foods, Pet Foods and Environmental Samples

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Introduction: Ready to use (RTU) media offers many advantages to the end user including less preparation and ease of use as well as being more sustainable. Easy Plate EC (Kikkoman Biochemifa Company) is a RTU device to enumerate *E. coli* and coliforms with greater selectivity compared to the traditional TBX and VRBLA.

Purpose: To compare the performance of the RTU device to those of ISO 16140-2: 2016 and ISO 4832:2006 for the enumeration of *E. coli* and coliforms in a broad range of foods, pet foods and environmental samples following the ISO 16140-2 validation protocol.

Methods: This study evaluated the specificity, selectivity, repeatability, accuracy and relative trueness of the RTU device as required by ISO 16140-2. During the study, 630 food samples were analyzed across seven categories; dairy products, fishery products, produce and fruits, meat and poultry products, multicomponent food, pet foods and environmental samples. All samples were tested according to the manufacturer's instructions, ISO 16649-2:2001 and ISO 4832:2006.

Results: Results from the study revealed good agreement between the RTU device and the reference method for both target organisms across the 210 samples analyzed in the relative trueness(Correlation coefficients are 0.984 for *E. coli* and 0.979 for coliforms). In the accuracy profile study, all seven categories tested satisfied the 0.5 log acceptability limit or the recalculated acceptability limits. Data also indicated that the RTU device was more selective and specific, differentiating more target and non-target organisms than TBX and VRBLA.

Significance: The RTU device gives comparative results to the reference methods ISO 16649-2:2001 and ISO 4832:2006 for a broad range of foods, pet foods and environmental samples. This RTU device is a convenient alternative for the enumeration of *E. coli* and coliforms.

P1-240 Evaluation of the Shimadzu Compact Dry CFR for the Rapid Detection of Coliforms in Raw Milk and Dairy and Heat Processed Dairy Products

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Introduction: Rapid enumeration of indicator organisms such as coliforms reduces time to result of hygiene monitoring in dairy products. These data aid the timely highlighting and subsequent resolution of potential contamination issues. Compact Dry CFR is a ready to use (RTU) media offering several advantages including ease of use, smaller incubation volume required, lower quantities of consumable waste and results within 18h post analysis.
Purpose: This study assessed the performance of the RTU media to enumerate coliforms in two categories; raw milk and dairy and heat processed dairy products. The evaluation followed the validation study design detailed in ISO16410-2:2016.
Methods: The performance of the rapid alternative method was compared to the reference method ISO 4832:2006 to assess method specificity, selectivity, robustness, accuracy, trueness and reproducibility.
Results: The relative trueness study results demonstrated good agreement between reference and rapid alternative methods across the 30 different dairy samples tested. Accuracy profile data met the required acceptability limits for the 60 products analyzed in the raw milk and dairy products and heat processed dairy products categories. Inclusivity and exclusivity study results showed the alternative method gave the anticipated results for all 50 inclusivity and 30 exclusivity isolates analyzed.
Significance: Study data demonstrate that the RTU media provides rapid, specific and selective enumeration of coliforms that has an equivalent performance to the standard reference method ISO 4832:2006 for raw milk and dairy and heat processed dairy products.

P1-241 Development of a Rapid Enrichment Broth for Injured *Escherichia coli* for Rapid Detection

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Introduction: *Escherichia coli*, causing public health problems, is managed with sodium hypochlorite (NaClO), a disinfectant, during food processing, yet detecting injured cells remains challenging.
Purpose: This study was conducted to develop a rapid enrichment broth for *E. coli* (REB-E) to rapidly recover and detect *E. coli* injured by NaClO.
Methods: REB-E was formulated by supplementing D-glucose, L-asparagine, and $MgSO_4$ to buffered peptone water (BPW) as carbon, nitrogen, and mineral sources, respectively, following the response surface methodology (RSM) and the modified Gompertz model. The optimal culture conditions for enhancing REB-E efficacy were determined at 34-40 ℃ and pH 6.31-7.91, and its impact was compared to BPW after 7 hours using tryptone soya agar (TSA). Cells were subjected to a 90-second treatment with a 10 ppm NaClO solution to induce injury, and the extent of damage was evaluated through plate counting on TSA and Eosin Methylene Blue (EMB) agar. Additionally, to validate the effectiveness of REB-E, the count of injured-*E. coli* was monitored through plate counting on TSA for 7 hours and confirmed through real-time PCR.
Result: REB-E was developed by supplementing BPW with 20 g/L D-glucose, 10.1 g/L L-asparagine, and 0.2 g/L $MgSO_4$, underwent optimization for culture conditions at 40℃ and pH 7.11. In the validation, the cell count increased by 1.1 log CFU/mL in REB-E compared to BPW after 7 hours, reaching a total growth of 6.31 log CFU/mL. *E. coli* experienced 73.333% injury in a 10 ppm NaClO solution. After 7 hours, the injured-*E. coli* enriched to 2.61 Log CFU/mL in EC broth, while in REB-E, they exhibited an increased growth up to 8.08 log CFU/mL. Furthermore, the real-time PCR results revealed that REB-E exhibited a Ct value of 15.79, approximately half that of EC broth.
Significance: REB-E developed in this study showed great potential in recovering and detecting injured-*E. coli* by NaClO.

P1-242 Method Comparison of Three Alternative Dehydrated Film Media for Total Plate Count, Coliform Count and *E. coli* and Coliform Count

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Introduction: Dehydrated film methods are often selected based on the assumption that if the method has completed the AOAC® *Performance Tested MethodsSM* (PTM) program, it will produce accurate test results across all sample types.
Purpose: The objective of this study is to determine if AOAC PTM certified methods for aerobic count, coliform count and *E. coli*/coliform count plates from three dehydrated film brands A, B and C are statistically different than the FDA BAM reference method across 5 different foods; paprika, flour, goat cheese with berries, frozen mixed fruit blend.
Methods: Five foods were weighed into 10-gram portions and serially diluted and pH adjusted. A medium target organism level of 50-100 CFU/g was used to spike samples using two cocktails containing *Escherichia coli* and *Klebsiella oxytoca* and *Staphylococcus aureu*s and *Bacillus spizizenei*. Samples were prepared at a 1:10 dilution and plated across dehydrated film brands A, B and C and plated onto FDA BAM reference media; Standard Methods Agar, Violet Red Bile agar and VRBA + MUG. Plates were incubated following AOAC PTM validated incubation time and temperature. Statical analysis was performed using a paired T-test. Acceptance criteria included results are not statistically different (p>0.05) based on paired-t test (95% CI) and the Mean Log Difference between all samples is < 0.5 log.
Results: Dehydrated brand A showed no statical difference compared to the refence method across all three plate types. Dehydrated film brand B showed no statical difference for aerobic count plate, however, was statically different for both coliform and *E. coli* and coliform dehydrated films. Dehydrated film brand C was statically different for all three plate types.
Significance: When selecting a dehydrated film method, it is important that the end-user performs a method verification of the method for each sample type to understand if that method is fit for purpose.

P1-243 Food Item Verification of Alternative Method Petrifilm Rapid *E. coli*/Coliform Count Plate and Reference ISO Methods for *Escherichia coli* and Coliforms Enumeration in Processed Meat

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Introduction: Rapid methods to enumerate coliforms and *Escherichia coli* for microbiological monitoring allow a quick response, supporting efficient quality control in food industries. Analysis of both coliform bacteria and *E. coli* are useful indicators of good processing practices in food industry. Even though Petrifilm Rapid *E. coli*/Coliform Count Plate is a validated method considered equivalent to the reference method for a broad range of foods, studying a new category of foods, "ready to cook meat products", had demonstrated that the alternative method is also applicable for this type of matrices.
Objective: This study aimed to study the performance of a rapid and ISO reference methods for quantification of coliforms and *E. coli* in ready to cook processed meat matrices.
Methods: Industrial samples of meat products formulations were tested, comprising 12 matrices of hamburger and meatballs. Natural contamination was used as interference microorganisms, and samples of each matrix were artificially contaminated with *E. coli* ATCC 8539 at low levels (5-10 CFU/g). Tenfold dilutions were plated on sample-ready or traditional media. Petrifilm Rapid *E. coli*/Coliform Plates and VRB plates were incubated at 37°C ± 1°C for 24 h, TBX plates were incubated at 44°C ± 1°C for 24 h. After incubation, plates were enumerated, and results were log transformed. For each sample, eBias was determined and accuracy profiles were obtained, to evaluate if eBias intervals (b-ETI) fall inside acceptability limits.
Results: Based on the data obtained, the alternative method is considered equivalent to the reference methods, for enumeration of coliforms and *Escherichia* coli in ready to cook meat products, specifically hamburger and meat ball matrices.

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Significance: The rapid method enabled reliable enumeration of coliforms and *E. coli* in processed food samples within 24 h for the category ready to cook meat products, with results comparable to ISO standards.

P1-244 Evaluation of Preparation Steps Used to Inoculate Almonds with Enterococcus faecium NRRL B-2354

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Introduction: *Enterococcus faecium* NRRL B-2354 (EF)-inoculated almond kernels, prepared with a standard method (SM), are used to validate reduction of *Salmonella* during thermal treatments.
Purpose: To assess steps in the SM for their impact on EF levels and on inoculated almond drying times.
Methods: Addition of yeast extract (YE) to tryptic soy agar (TSA) or broth (TSB), incubation times, volume for seeding agar lawns, or inoculum volume per 400 g of almonds were assessed. An isolated colony from TSA without (SM) or with 0.6% YE was transferred into corresponding TSB (SM) or TSBYE, incubated at 37°C for 24 h, transferred into fresh broth, and incubated at 37°C for 18 h (SM) or 24 h. The 18-h (1 mL; SM) or 24-h culture (0.25, 0.5, or 1 mL) was spread over corresponding TSA (SM) or TSAYE plates (150-mm) and incubated at 37°C for 24 h. Cells were collected in 6 mL of peptone; 25 mL (SM) or 4, 10, or 15 mL of inoculum was added to 400 g of almonds, which were then dried at ambient temperature to original moisture levels. EF populations in the inoculum and on the almonds were determined using standard methods.
Results: Compared to the SM, EF populations in the inoculum suspension were not significantly ($p \geq 0.05$) impacted by addition of YE to either broth or agar or by the amount of culture used to seed bacterial lawns but were significantly ($p < 0.05$) larger ($\Delta = 0.33$ log) when the incubation time was increased to 24 h; populations of EF on dried inoculated almonds were significantly lower at 4 (7.43 log CFU/g) and 10 (7.95 log CFU/g) but not at 15 mL (8.25 log CFU/g) of added inoculum and a significant 24-h reduction in drying time was achieved.
Significance: Small modifications in the SM lead to increased final populations of EF on almonds with a corresponding decreased drying time.

P1-245 Absolute Quantification of *Campylobacter jejuni* in Raw Chicken Breast

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❖ ***Developing Scientist Entrant***

Introduction: Enumeration of foodborne pathogen *Campylobacter jejuni* in poultry products is exigent. However, quantifying *Campylobacter* spp. with qPCR has been constrained due to the high limit of detection (LOD), 4 log CFU/mL, associated with these assays. An alternative technology, Nanoplate dPCR, provides enhanced sensitivity and absolute quantitation.
Purpose: Establish *Campylobacter jejuni* absolute quantification method for raw chicken breast using a modified Nanoplate dPCR assay.
Methods: *C. jejuni* (ATCC 33560) was plated on Campy Chromogenic Agar (CCA) and incubated under microaerophilic conditions for 24-h at 42°C. One colony was transferred to Bolton Broth (10mL) and incubated under the same conditions. The culture was washed twice and resuspended in 10mL of 0.1% Buffered Peptone Water (BPW). The resuspended culture was diluted (1:10) to 10^{-10} and spread plated on CCA to determine *C. jejuni* level. Culture was confirmed to contain 3.28×10^7 CFU/mL of *C. jejuni*. Dilutions, ranging from 328 to 0.00328 CFU/mL, were inoculated (1mL) onto raw chicken breast (25g) in a filtered homogenizer bag. Samples were homogenized in 99mL of 0.1% BPW for 90-s at 230rpm. DNA extractions were performed using the Qiagen Blood and Tissue Kit Gram-Negative protocol. Detection was carried out using a Nanoplate dPCR system and respective Microbial DNA primers and probes. Count data were calculated as Log CFU/g of chicken, and data was analyzed using linear regression ($p \leq 0.05$).
Results: A linear fit equation was generated using absolute values (copies/mL and CFU/g) to determine the fit of the data ($y = 0.079 + 0.004\times$), yielding an R^2 of 0.86 and RMSE of 0.16. LOD was 0.734 CFU/g, or less than one cell ($p < 0.001$).
Significance: Nanoplate dPCR can quantify foodborne pathogens such as *Campylobacter jejuni* at levels lower than 0 CFU/g (0.734 CFU/g). This study demonstrates that NanoPlate dPCR is a highly sensitive and absolute quantitative technology for foodborne pathogen detection in comparison to qPCR.

P1-246 Quantification of Viable but Non-Culturable Campylobacter jejuni by Using PMA-qPCR and Dielectrophoresis with Micro-Fluidic Device

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Introduction: One of the causes of foodborne illness by *Campylobacter jejuni* may related to existence of viable but non-culturable (VBNC) cells induced by exposure to various environmental stresses. Rapid quantification of the VBNC state *C. jejuni* cells plays a key role in appropriate risk assessment and developing clear control measures.
Purpose: The objective of this study was to investigate the environmental conditions, in particular desiccation, for inducing VBNC state of *C. jejuni* and to quantify the number of VBNC cells using PMA-qPCR and dielectrophoresis (DEP) with micro-fluidic device.
Methods: An aliquot of 10 µL pre-grown *C. jejuni* cells (10^8 CFU/mL) were dried at 25°C and 50%RH in an aerobic condition to investigate the occurrence of VBNC cells during drying. The culturable bacterial cells were enumerated by mCCDA culture media, and the VBNC cells were quantified by PMA-qPCR method as a standard procedure and DEP procedure using the apparatus (ELESTA PixeeMo, AFI Corp., Japan) with 3 to 7 MHz frequencies.
Results*:* Although culturable *C. jejuni* cells were gradually decreased during drying process and not detected (<10^1 CFU/mL) after 12 h at 25°C drying, the live cell numbers were detected >4 log cells/mL by PMA-qPCR method. In addition, live cells were observed by fluorescence microscopy. Likewise, *C. jejuni* cell numbers enumerated by the DEP method with 5 MHz frequency illustrated >4 log cells/mL after 12 h drying, which is similar to the results of the PMA-qPCR method. In contrast, no *C. jejuni* cell numbers were detected by the DEP method with 7 MHz frequency, illustrating equivalency to the results of culture method.
Significance: The combined technique of DEP with micro-fluid device suggests that the possibility of detection and quantification of the number of VBNC *C. jejuni* without chemical labelling as used in PMA-qPCR and fluorescence microscopy observation.

P1-247 Development of a Portable Method for Single-Tube Capture, Concentration, and Genomic Extraction of Tulane Virus, a Human Norovirus Surrogate, Using Magnetic Ionic Liquids

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Introduction: Capture and concentration of viruses from contaminated foods is crucial for sensitive detection. Magnetic ionic liquids (MILs) are ideal capture reagents for portable applications since they require minimal equipment and no cold storage. However, they would need a similarly portable genomic extraction method to be used with existing nucleic acid-based detection methods. Since MILs effectively bind both intact virus and viral RNA, they could potentially be used as binding substrate for both viral capture and genomic extraction.
Purpose: To develop a fully portable sample preparation method that combines target capture, concentration, and genomic extraction in one tube.
Methods: Tulane virus, a human norovirus surrogate, was diluted into PBS to 10^5 PFU/mL and captured using cobalt, nickel, or dysprosium-based MILs. MILs were combined with viral suspension, vortexed, and magnetically separated. Unbound virus was removed by water rinse and captured virus was eluted from MILs using modified Luria broth. Viral RNA was extracted using Trizol and quantified by RT-qPCR.
Results: Tulane virus adhered strongly to experimental vials, displaying 10.0±1.72% (n=3) recovery even when no MILs were used. This was not ob-

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served in earlier studies with bacteriophages and may be due to some component of the storage media. Adding 0.05% Tween-20 to the viral suspension significantly (p<0.05) mitigated this, reducing control recovery to 0.47±0.65% (n=3). Nickel- and dysprosium-based MILs maintained efficacy in the presence of Tween, displaying 22.3±15.3% and 90.0±5.20% capture efficiency and 25.0±3.11% and 14.9±2.50% recovery efficiency, respectively (n=3). Optimizing dispersion times helped increase this further. Cobalt-based MILs lost efficacy in the presence of Tween; therefore, further studies will use only nickel- and dysprosium-based MILs.

Significance: MILs displayed strong affinity for Tulane virus, and the apparent impact of viral storage media has implications for the use of MILs in complex matrices. Next steps will involve developing viral genomic extraction reagents that are compatible with MILs.

P1-248 Methods Evaluation for Concentration of Viruses in Large Volumes of Agricultural Water

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Introduction: Foodborne viruses are major causes of enteric outbreaks, which often are associated with fresh produce. Virus surveillance in agricultural water can help understand the sources and levels of contamination during production and processing. Due to the low levels of viruses in the environment, large-volume sampling with multi-step concentration is required for detection.

Purpose: The objective of this study was to evaluate two methods, dead-end hollow fiber ultrafiltration (DEUF) and modified Moore swab (MMS), for sample collection and concentration of enteric viruses from 10 litres of agricultural water. For secondary concentration methods, polyethylene glycol (PEG) precipitation and ultracentrifugation were used for comparison.

Methods: REXEED-25S ultrafilters and MMS were utilized in capture-filtration of viruses in the water. Human norovirus (NoV) as a representative for unenveloped viruses and murine hepatitis virus (MHV) for enveloped viruses were co-spiked into stream water (pre-filtration) or eluate after filter elution (post-elution). Viruses were further concentrated by either PEG precipitation for DEUF or ultracentrifugation for MMS samples following initial elution. RNA was extracted with guanidinium thiocyanate (GITC) and QIAGEN RNeasy kit. Performance of different methods was assessed using Ct values generated by individual RT-qPCR for each virus.

Results: When viruses were spiked prior to filtration, the Ct values of both NoV and MHV were consistently lower with DEUF than MMS at all detectable levels (Ct 29.9 - 42.0), e.g. Ct differing by more than 4.5 cycles at the highest seeding level (p<0.05), demonstrating a better performance of the DEUF method in virus recovery. Using post-elution spiked viruses, the Ct values of both viruses (Ct 25.1 - 41.9) showed no significant difference between PEG precipitation and ultracentrifugation (p>0.05), suggesting PEG precipitation is an effective, easy-to-use alternative to ultracentrifugation for virus concentration, especially for large volumes of water.

Significance: The method development toward multi-virus detection in agricultural water provides the scientific basis and practical tools for the prevention and investigation of foodborne illnesses.

P1-249 Evaluation of qPCR Parameters for Use in the FDA Bacteriological Analytical Manual for *Cronobacter* Detection

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Introduction: *Cronobacter* spp., a group of Gram-negative pathogenic bacteria, poses a significant risk to infants and premature births. Naturally occurring in the environment, *Cronobacter* can persist in low-moisture foods like powdered infant formula (PIF). Chapter 29 of the FDA Bacteriological Analytical Manual (BAM) outlines the official method for *Cronobacter* detection in PIF, including culturing and qPCR portions.

Purpose: With rapid advancement of technologies, there is a need to assess key aspects of the qPCR method to continuously update the BAM, ensuring consistent support for the *Cronobacter* program.

Methods: Five *Cronobacter* qPCR parameters, including the probe quencher, polymerase, magnesium concentration, annealing temperature, and internal amplification control (IAC), were evaluated using genomic DNA of pure cultures and extracts of spiked PIF, with primer and probe sequences remaining unchanged. Cycle threshold values and intensity of the end-point amplification signals were used to optimize the qPCR.

Results: By comparing the fractional positive rates of qPCR results, optimized parameters were successfully determined. The ZEN double-quencher yielded a signal-to-noise ratio approximately 1.5-times of the TAMRA quencher, with similar background levels and end-point signals at 10 and 6 fluorescence units, respectively. Additional Platinum Taq (2.5 U per reaction) and 3 mM $MgCl_2$ were used in the qPCR. The optimized annealing temperature range was determined to be 52 to 56 °C, showing approximately 10 times great sensitivity than 60 °C using the same spiked test samples. For internal amplification control (IAC), a synthetic dsDNA fragment and a pUC57-based plasmid (both at 3000 copies per reaction) achieved satisfactory target cycle threshold values between 24 and 34 following the quality requirement.

Significance: As analytical results offer critical scientific evidence for regulatory decision-making, this work makes significant contribution to the continuous improvement of *Cronobacter* qPCR, aligning well with FDA's Prevention Strategy to mitigate *Cronobacter*-related foodborne illnesses.

P1-250 Evaluation of Reduced-Rehydration Methods for the Detection of *Cronobacter* in Increased Sample Size of Powdered Infant Formula

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Introduction: In order to improve the efficiency of testing for *Cronobacter* in contaminated powered infant formula (PIF), larger sample sizes are suggested. However, potential osmotic shock to desiccated *Cronobacter* cells is a concern when PIF is rehydrated with large volumes of liquid media, and thus, the use of reduced volumes of liquid media was evaluated for rehydration and enrichment.

***Purpose*:** This study investigates the effect of increasing sample size to 400 g while employing reduced rehydration using pre-warmed media to minimize potential osmotic shock during the isolation of *Cronobacter* cells in PIF.

***Methods*:** Lyophilized *Cronobacter* cells were spiked in 400 g of PIF products at 0.25 to 2 CFU per test portion, which was subsequently rehydrated and incubated in buffered peptone water (BPW) pre-warmed at 36°C. Two types of reduced-rehydration methods were evaluated: 1) 1:3 sample/broth ratio for 1 h initial rehydration and then 1:10 sample/broth ratio for the remaining 23±2 h incubation; and 2) 1:5 sample/broth ratio during the entire 24 ± 2 h incubation, which were compared to the traditional 1:10 sample/broth ratio during the entire incubation, as described in the FDA *Bacteriological Analytical Manual* (BAM). Multiple trials were performed for each comparison.

***Results*:** The reduced-rehydration using pre-warmed BPW and a 1:3 sample/broth ratio for 1 h followed by 1:10 ratio showed no difference from the 1:10 sample/broth ratio (26/40 vs. 25/40) in the detection of *Cronobacter*; likewise, the reduced-rehydration with 1:5 sample/broth ratio showed no difference from the 1:10 sample/broth ratio (11/40 vs. 9/40).

Significance: The data indicated that reducing the volume of liquid media did not improve the recovery of *Cronobacter* in large sample sizes of PIF when compared to the BAM rehydration and enrichment method for the recovery of *Cronobacter,* which suggests the feasibility of increasing sampling sizes for detecting *Cronobacter* in PIF.

P1-251 *Cronobacter* Morphologies on Four Selective Chromogenic Agars: An Inclusivity Study Using 380 *Cronobacter* Isolates

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Introduction: In response to the emerging *Cronobacter* infections among infants in the United States, commercially available chromogenic selective agars for isolating *Cronobacter* were evaluated for possible inclusion in the FDA *Bacteriological Analytical Manual* (BAM).

Purpose: Four types of selective chromogenic agars were evaluated for the detection of *Cronobacter* using 380 *Cronobacter* isolates and 53 non-*Cronobacter* isolates.

Methods: Frozen isolates were activated in brain heart infusion broth and subsequently streaked onto selective agars: 1) Brilliance *Enterobacter sakazakii* agar (DFI). 2) *Enterobacter sakazakii* chromogenic plating agar (R&F). 3) Chromogenic *Cronobacter* Isolation agar (CCI). 4) *Enterobacter sakazakii* Isolation Agar (ESIA). The agars were incubated at temperatures recommended by their manufacturers (DFI at 36±1°C, CCI at 41.5±1°C, R&F at 36±1°C and 41.5±1°C, ESIA at 44±1°C) for up to 48 h and the morphologies of colonies were examined.

Results: Significant variations were observed in the color and morphology of colonies of different *Cronobacter* isolates on the selective agars, which we categorized into several major groups. For example, green, pale-green, and yellow-green, etc. for DFI and CCI agar; blue-grey, blue-black or light-grey, etc. for R&F agar; and blue-green, light-blue, etc. for ESIA agar. Morphology of some isolates fell outside these major categories. Co-growth of *Cronobacter* and non-*Cronobacter* on the same agar plate revealed apparent color-shifts of *Cronobacter* colonies, for example, black-colored colonies may appear green after background flora change the agar color from red to yellow. Such phenomena are noted in the guidance. Panels of colony morphologies were constructed to provide guidance for *Cronobacter* isolation.

Significance: The study analyzing 380 *Cronobacter* isolates on four types of selective chromogenic agars revealed broad variety of colony morphologies. The updated BAM chapter with additional options for selective agars or incubation temperatures enhances the isolation of *Cronobacter* on chromogenic agars.

P1-252 Next-Day Detection of *Cronobacter* Species in Powdered Infant Nutritionals, Milk Powders and Environmental Samples Using the Assurance® GDS for *Cronobacter* Tq II Assay

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Introduction: *Cronobacter* is a pathogen of concern in infant nutritionals. An extension for a new rapid confirmation method for Assurance® GDS for *Cronobacter* Tq II was performed (Campden BRI, Expert Lab) in the food category of infant formula and infant cereals, including milk powder. This configuration was established by MicroVal, where an inclusivity and exclusivity study and a reduced sensitivity study are used to assess a new confirmation method to an existing qualitative method.

Purpose: To validate the next-day rapid confirmation of *Cronobacter* in infant nutritionals, milk powder, and environmental samples compared to the ISO reference method.

Methods: The data included 46 samples for the sensitivity study and 100 strains for both inclusivity and exclusivity. Lyophilized cultures of *Cronobacter* were inoculated into foods and stabilized at room temperature for a minimum of 2 weeks. Samples (375g) were enriched 1:10 in the appropriate media for 24 h and rapidly confirmed by either direct streak or Immunomagnetic separation (IMS) onto 3 chromogenic agars (2 agars for IMS). All inclusivity and exclusivity isolates were streaked onto all chromogenic plate types, followed by MALDI ToF and biochemical analysis of typical colonies.

Results: The findings obtained through the direct streak and IMS methodologies employed in the isolation and confirmation of *Cronobacter* demonstrate favorable concordance within the context of the sensitivity study, encompassing 46 tested samples, all of which exhibited results falling below the stipulated Acceptability Limit (AL).

Significance: This method comparison study gathers data according to the MicroVal interpretation guidelines for ISO 16140-Part 6 (2019) to validate an extension study for a new confirmation method from a previously validated qualitative method. The new confirmation methods provide expanded methods for rapid confirmation for *Cronobacter* and are selective and specific following Assurance® GDS for *Cronobacter* Tq II detection method.

P1-253 Evaluation of Real-Time PCR for the Detection of *Cronobacter* in Powdered Infant Formula

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Introduction: *Cronobacter* is a foodborne pathogen that has been linked to powdered infant formula (PIF) contamination. Real-time polymerase chain reaction (qPCR) enables rapid and precise detection of *Cronobacter*, but DNA extracted from PIF samples could cause high fluorescent background noises in qPCR.

Purpose*:* The qPCR method in the *Cronobacter* chapter in the 2012 FDA *Bacteriological Analytical Manual* (BAM) for detecting *Cronobacter* in PIF samples was evaluated with a focus on sensitivity and background normalization.

Methods*:* For the first modification, a passive reference dye was added to the mastermix to normalize high background noise generated in the PIF samples. Second, a manual threshold was established to ensure that background noises do not cause false positive results. Third, the baseline calling rule was modified to avoid baseline calling errors. The sensitivity of the modified qPCR protocol was compared to the original qPCR protocol. *Cronobacter* was artificially inoculated into PIF rehydrated in buffered peptone water (BPW) to reach a level of 9 log CFU/mL. Serial dilutions were performed using a blank PIF sample without *Cronobacter* inoculation to produce various levels of DNA used to determine the limit of detection of the qPCR reaction. The revised method was performed on DNA extracted from pure cultures of 66 *Cronobacter* strains in an inclusivity testing and 45 non-*Cronobacter* species in an exclusivity testing.

Results*:* The addition of a passive reference dye to the mastermix reduced the high background fluorescent noise caused by PIF samples while maintaining the high sensitivity. The revised qPCR was able to detect 5-10 genome copies per reaction, while making sure background fluorescent noises stay below the thresholds. Inclusivity and exclusivity panels demonstrated that the revised qPCR is highly specific to *Cronobacter*.

Significance: The revised qPCR method is highly sensitive and specific. In addition, it ensures that any fluorescent background noises do not cause false positive results.

P1-254 Development of a Novel CrAss-Like Phage Detection Method with a Broad Spectrum for Microbial Source Tracking Marker

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Introduction: CrAssphage, a highly abundant bacteriophage in the human gut, has been used as a microbial source tracking (MST) marker. Recently, many crass-like phages have been discovered to expand the classification into new order *Crassvirales*.

Purpose: This study aims to assess crAss-like phage prevalence in South Korea and develop a detection system for MST applications.

Methods: Fifty-nine human fecal samples (ages 19 to 45) and twenty-one animal fecal samples from six species (deer, wild birds, wild cats, raccoons, dogs, and cats) were collected in Korea. The virus-like particles (VLPs) were extracted and sequenced on the Illumina NextSeq 2000 platform. CrAss-like phages were annotated using Prokka v1.14.6, and the whole genome sequences (WGS) were compared using tBLASTx in Easyfig (version 2.2.5). Sequence similarities of WGS and major head protein (MHP) were calculated using the Virus Intergenomic Distance Calculator (VIRIDIC). PCR primer candidates, designed using NCBI's Primer-BLAST tool, were screened in human and animal feces.

Results: Thirteen crAss-like phages were identified in six human fecal samples, forming seven groups through metagenomic analysis. Major head protein (MHP) exhibited higher sequence homology within each group. Eight PCR primer candidates, designed from MHP sequences, were evaluated in animal and human feces. CrAss-like phages were not detected in animal feces except in raccoons, hosts of groups VI, VIIa, and VIIb. CrAss-like phages were detected in 91.52% (54/59) of humans, and group VI (38/59) exhibited the highest prevalence, nearly twice that of p-crAssphage in group I (22/59).

Significance: This study emphasizes group VI as an effective MST marker, expanding the detection range for crass-like phages. Human-specific and selective MST markers can significantly influence hygiene regulations, reducing public health costs through disease screening and source tracking.

P1-255 Detection and Enumeration of Total Viable Bacteria from Various Surfaces using Hygiena's MicroSnap SX-TVC (MicroSnap SX(Surface Xpress) for Total Viable Count) and Comparison to the Standard ISO 18593:2018 Method

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Introduction: Many foodborne illnesses originate from contamination of uncleaned environmental surfaces and manufacturing equipment. The MicroSnap SX-TVC (Surface Xpress for Total Viable Count) is an all-in-one rapid bioluminogenic test for enumeration of Total Viable Bacteria from environmental surfaces.

Purpose: To demonstrate detection of Total Viable Bacteria from 5 different surface matrices and comparison to the ISO 18593:2018 method in an unpaired study.

Methods: Stainless-steel, concrete, plastic, ceramic, and rubber coupons (100 cm^2) were inoculated with *S. aureus*, *B. cereus, S.* Typhimurium*, L.* monocytogenes*,* and *E. coli,* respectively, at four different CFU levels and then allowed to dry for 18-24 h. After drying, the samples were collected from the surfaces using MicroSnap SX-TVC; the devices were incubated at 32 °C and tested at 4 and 5 hours, respectively. BAM Ch.3 plus ISO 18593:2018 methods were run alongside these validations in an unpaired study to determine the recovered CFU levels from the surface post-drying.

Results: The CFUs gathered by MicroSnap SX-TVC were collected at 4 h and 5 h, respectively, and compared with BAM Ch. 3 plus ISO 18593:2018 to determine equivalency. The 4-hour results showed that amongst the four different spike levels (10-100, 100-1,000, 1,000-10,000, & 0 CFU), the difference of mean between the two methods were within half a log of each other, and the 90% confidence interval fell within the equivalency range. The 5-hour incubation results show us that most spiked levels fell within the equivalency range and the difference of means were within half a log to the reference method, confirming that the two methods are similar for detection of total viable bacteria from the matrices.

Significance: The MicroSnap SX-TVC can be used as a simple, rapid, and accurate method to measure Total Viable Bacteria from the five different matrices as compared to traditional methods.

P1-256 Detection and Enumeration of *Enterobacteriaceae* Organisms from Various Surfaces Using Hygiena's MicroSnap SX-EB (Surface Xpress for *Enterobacteriaceae*) and Comparison to the ISO 21528-2:2017 Method

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Introduction: Many foodborne illnesses originate from contamination of uncleaned environmental surfaces and manufacturing equipment. The Enterobacteriaceae group of gram-negative organisms is often used as an indicator of fecal contamination and contains a number of harmful bacterial species. The MicroSnap SX-EB (Surface Xpress for *Enterobacteriaceae*) is an all-in-one rapid bioluminogenic test for enumeration of *Enterobacteriaceae* from environmental surfaces.

Purpose: To demonstrate detection and enumeration of Enterobacteriaceae in less than 6 hours from 5 different surface matrices and compare results with ISO 21528-2:2017 method in an unpaired study.

Methods: Stainless-steel, concrete, plastic, ceramic, and rubber coupons (100 cm^2) were inoculated with *E. aerogenes/S. aureus (as a non-target competitor)*, *C. freundii, S. abaetetuba, C. malonaticus,* and *E. coli* respectively at four different CFU levels and then allowed to dry for 18-24 h. After drying, the samples were collected from the surfaces using MicroSnap SX-EB and the devices were incubated at 37 °C and tested at 4 and 5 hours, respectively. The ISO 21528-2:2017 method was run alongside this validation as an unpaired study to determine the recovered CFU levels from the surfaces post-drying.

Results: The CFU gathered by MicroSnap SX-EB was collected at 4 h and 5h, respectively, and compared with the ISO 21528-2:2017 results to determine equivalency. The 4 and 5-hour results showed that amongst the four different spike levels (10-100, 100-1000, 1000-10000, & 0 CFU), the difference of means between the two methods were within a half log of each other, demonstrating very similar CFU counts from both methods.

Significance: The MicroSnap SX-EB can be used as a simple, rapid, and accurate method to measure *Enterobacteriaceae* from the five different matrices as compared to a traditional method.

P1-257 Evaluation of a Targeted Amplicon Sequencing Method for Detection of Contaminating Microorganisms in a Probiotic Product

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Introduction: In recent years there has been an increase in consumption of live microbial dietary supplements or probiotics. One of the challenges in maintaining the safety of probiotic products is the detection of low levels of contaminating microbes and/or pathogens in the presence of large number of beneficial microbes. Whole metagenomic sequencing (WMS) and target amplicon sequencing (TAS) has proven to be robust methods for detection of pathogens in food matrices. Here we evaluate and compare how accurate and sensitive these two sequencing methods are in detecting low levels of microbial contaminants in a probiotic matrix.

Purpose: The aim of this work is to determine the lowest level of spiked *Listeria monocytogenes* in a probiotic product that can be detected accurately by WMS and TAS.

Methods: A probiotic product which contained only one bacterial species, *L. rhamnosus,* was chosen to spike with the foodborne pathogen, *L. monocytogenes* (ranging from $2x10^9$ to 20 CFUs). The sensitivity of detection using TAS was evaluated against WMS. Target amplification was done using a custom primers panel which includes ten *L. monocytogenes* genes and eight *L. monocytogenes* virulence genes. Respective library preparation methods for TAS and WMS were used, and the libraries were sequenced on the Illumina MiSeq platform. GalaxyTrakr and BLAST matching of the amplicons were used for data analysis.

Results: *L. monocytogenes* could be detected by WMS as low as 20,000 CFU while using TAS it could be detected as low as 20 CFU at the species, strain, and virulence genes level.

Significance: Detecting low concentrations of contaminating microorganisms amidst the abundant background of probiotic microorganisms along with

the added probiotic matrix poses a challenge in DNA isolation and DNA based detection. We show that TAS is a sensitive method for detection of low-level contaminants that may be present in probiotic products thus enhancing public health safety.

P1-258 Validation of a Ready-to-Use *Staphylococcus aureus* Test Compared to FDA-BAM and ISO Methods

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Introduction: Microbial testing platform, Peel Plate (PP), was developed with Baird Parker ingredients for *Staphylococcus aureus* (SA) specificity. Rehydrated with 1 mL of food dilution, PP-SA are incubated 24-48 hours at 35-37°C and observed for purple colonies.

Purpose: Validate PP-SA with inclusivity/exclusivity study and comparison of spiked samples to reference methods using selected non-cultured food matrices.

Methods: PP-SA (Charm Sciences, Inc.) were evaluated by Charm and Q-laboratories: 38 non-SA strains and 50 SA strains at 100 CFU/mL were from ATCC and Q-labs. Non-fat dried milk, cod filet, infant formula, vegetable soup, frozen shrimp, fr.vegetables, potato salad, corned beef were spiked at low, medium, and high levels spanning 1-3 log CFU/g with *Staphylococcus aureus* strains and allowed to acclimate dried 2 weeks, refrigerated 4°C for 72 hr, or frozen 2 weeks at -20°C. Unfortified and spiked levels were tested (N=5) by PP-SA in duplicate and reference methods, FDA-BAM Chapter 12 or ISO 6888-1:2021 methods. 50g portions were rehydrated with 450 mL diluent for BAM method and 25g portions in 225 mL diluent for ISO method. Paired analysis for log differences and 90 and 95% confidence limits were performed as developed by LaBudde.

Results: PP-SA excluded all non-SA strains and detected 49 of 50 SA strains. Log mean levels for all evaluated foods were not significantly different from BAM method means as defined by log differences lower 95% confidence level (LCL) not less than -0.5 log and upper level (UCL) not greater than 0.5 log. ISO method mean levels were not significantly different, except mid-level cod fish PP-SA LCL . 24-hour SA growth was observed in all matrixes except one cod fish filet perhaps due to whitener additive.

Significance: Peel Plate *Staphylococcus aureus* is an alternative ready-to-use method for detection of SA in a variety of non-cultured foods. As with reference methods, PP-SA colonies should be isolated and confirmed catalase and coagulase positive.

P1-259 An Interlaboratory Study on the Detection Method for an Emerging Enteropathogen of *Escherichia albertii* in Food

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Introduction: *Escherichia albertii* is an emerging enteropathogen and its outbreak of foodborne infection was reported in Japan; however, systematic procedures for its specific detection in foods have not yet been established.

Purpose: To establish a series of effective methods for detecting *E. albertii* in food, an interlaboratory study was conducted.

Methods: Nine chicken and bean sprout samples were prepared as follows: three samples each were inoculated at a low level (17.7 CFU/25 g), high inoculation level (88.5 CFU/25 g), and uninoculated. Samples were enriched in modified EC broth supplemented with cefixime and tellurite followed by *E. albertii* specific real-time PCR assay (EA-rtPCR) and plating on deoxycholate hydrogen sulfide lactose agar (DHL), MacConkey agar (MAC), and these agars supplemented with rhamnose and xylose (RX-DHL and RX-MAC). EA-rtPCR was performed to noncolored colonies to identify *E. albertii* colonies. Data of inoculated and uninoculated samples were expressed as sensitivity (the number of positive samples divided by the total number of inoculated samples) and specificity (the number of negative samples divided by the total number of uninoculated samples), respectively.

Results: The sensitivity of EA-rtPCR was 1.000 for chicken and bean sprout samples inoculated with *E. albertii* at low and high inoculation levels. With selective agars supplemented with RX, *E. albertii* at an inoculation level of over 17.5 CFU/25 g of food was detected with a sensitivity of 1.000 and 0.667–0.727 in chicken and bean sprouts, respectively. In bean sprouts inoculated with *E. albertii*, EA-rtPCR was significantly superior to plating on DHL and MAC. Plating on RX-DHL and RX-MAC was significantly superior to plating on DHL and MAC in chicken samples, and to plating on MAC in bean sprouts samples.

Significance: Screening for *E. albertii*-specific gene using EA-rtPCR followed by isolation with RX-DHL or RX-MAC is an efficient method for *E. albertii* detection in food.

P1-260 Development of an Accurate and PCR-Independent Identification Method for *Bacillus cereus* Group Using a Nanopore-Based Sequencer and Genome Database

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Introduction: Although *Bacillus cereus* group consists of *B. thuringiensis*, *B. anthracis*, and *B. cereus*, the latter of which is a food pathogen, it is hard to discriminate these species due to extraordinarily high similarities of their 16S rDNA sequences.

Purpose: The objective of this study is to develop a new method for distinguishing the species belonging to *Bacillus cereus* group by direct analysis of genomic DNA.

Methods: The genomic DNA of *B. cereus* and *B. anthracis* were purchased. The other strains of *B. cereus* group were purchased and then cultured in liquid and/or agar media. Identification of these strains were performed by MALDI-TOF MS and 16S rDNA sequencing. In addition, genomic DNA sequence data from these strains were obtained about 1000 reads using MinION (Oxford Nanopore Technologies). Then, the obtained sequence data (FAST5 format) were base-called by guppy-GPU (ONT), and the identification results were visualized as Krona chart using the GenomeSync database and GSTK (genome search toolkit) system.

Results: MALDI-TOF MS was unable to discriminate strains among the *B. cereus* group. Among the thirteen *B. cereus* group strains, only *B. mycoides* strains were distinguished by 16S rDNA sequencing, whereas the gDNA-GSTK method was able to correctly identify all the strains at the species level. Therefore, the gDNA-GSTK method was considered as the most accurate method for identifying *B. cereus* group strains among three methods evaluated: MALDI-TOF MS, 16S rDNA sequencing, GSTK. Additionally, the developed method does not require PCR amplification of genes and is applicable to any microbial species. Furthermore, by using this method, identification of microbes was possible within 30 minutes after obtaining genomic DNA from the microbial strains, significantly reducing the time required for DNA analysis.

Significance: These results suggest that this method is one of the effective means of quality control in the food industry.

P1-261 Investigation of Pathogenic Bacterial Response to Chemical Stimuli Using Concentric Wrinkled as Confined Culture Volume

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Introduction: The dynamic behavior of microorganisms may appear differently in micro-sized structures than in a general environment.
Purpose: This study explores the response of pathogenic bacteria, specifically *Escherichia coli* O157:H7 and *Bacillus cereus*, to chemical stimuli within a confined culture volume.
Methods: Concentric micro-wrinkle structures were fabricated by inducing contraction of voids in a rubber matrix using PDMS. An oxide thin film was formed through oxygen plasma treatment, and a micro-sized wrinkle structure was formed on the PDMS surface. The microbial strains, *E. coli* O157:H7 and *B. cereus*, were cultivated within confined volume using the manufactured structure and flat cover glass. Chemical stimuli such as IPTG were introduced into voids in PDMS matrix, allowing for real-time observation of microbial reactions using fluorescence microscope.
Results: The study confirmed that the arrangement of pathogenic bacteria within concentric micro-wrinkle structures is influenced by the aspect ratio during cultivation. In the case of *E. coli*, it was observed that bacterial cells were arranged according to a pattern of the micro-sized PDMS wrinkles during 24 hr culture. At the beginning of culture, *E. coli* moved along the wrinkles and when two *E. coli* bacteria meet in one wrinkle, the other bacteria is pushed out by the bacteria with great force. The result of examining the behavior of microorganisms through chemical stimulation by injection of IPTG solutions of different concentrations into the voids showed that the growth of bacteria was inhibited at 1M ITPG concentrations. And at 0.1M concentrations, GFP protein expression increased and growth and reproduction also occurred well. In the case of BL21 bacteria, IPTG stimulation allowed single cells to grow to a length of more than 100 μm.
Significance: This knowledge has implications for designing novel strategies using the developed concentric micro-wrinkle structures offer a platform for studying pathogenic microbial behavior, chemical resistance, and intermicrobial interactions.

P1-262 Development of Novel Analytical Tools for the Rapid Detection of Micro-and Nano-Plastics in Agri-Foods

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Introduction: The increasing accumulation of micro-and nano plastic in agri-food systems poses tremendous risks for terrestrial and aquatic organisms as well as human health.
Purpose: We aim to develop novel and easy-to-use analytical tools for the rapid, reliable, and on-site detection of micro-and nano-plastics in agri-foods
Methods: We used luminescent metal-phenolic networks (L-MPNs) labeling as dual tags for establishing both fluorescence and surface-enhanced Raman scattering (SERS) sensing platforms. L-MPNs were composed of zirconium ions, tannic acid, and rhodamine B to label a wide range of plastic particles. Fluorescence-enabled portable device and SERS techniques were employed for the rapid screening and accurate determination of various sizes (50 nm-10 μm) and types (e.g., polystyrene, polypropylene, polyethylene terephthalate) of micro-and nano-plastics in real-world agrifood settings.
Results: Results showed that the portable device can quantify micro-and nano-plastics as low as 330 microplastics and 3.08×10^6 nanoplastics in less than 20 min. Moreover, the device is low cost ($0.015 per assay), user-friendly, and operative by untrained personnel to conduct data processing on mobile device APP remotely. The SERS platform enabled the sensitive detection of nanoplastics with a limit of detection of 0.1 ppm. We also demonstrated the applicability of the sensing platforms to real-world samples through the determination of micro-and nano-plastics released from plastic cups after hot water and flow induction, and nanoplastics in tap water.
Significance: The developed analytical tools can quickly and accurately detect various types of plastics with micro or nano sizes with minimal sample preparation and high throughput in laboratory and non-laboratory settings. The outcome of this study will benefit the long-term safety and sustainability of agrifood systems.

P1-263 Rapid On-Site and Sensitive Detection of Microplastics Using Zirconium (IV)-Assisted SERS Label

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❖ ***Developing Scientist Entrant***

Introduction: Microplastics have emerged as significant pollutants in both terrestrial and marine ecosystems, posing a threat to human health, making their accurate identification and quantification essential.
Purpose: In this study, we aim to develop the rapid onsite and sensitive detection of polystyrene microplastics by Zr^{4+} assisted RhB labeling method.
Methods: Zirconium ions (20 mM) were used to connect rhodamine B (60 mM) to polystyrene microplastics (10 μm) through cation-π interactions, in a process termed the Zr^{4+}-assisted RhB labeling strategy. This strategy, combined with surface-enhanced Raman spectroscopy (SERS) using an AgNPs substrate (54 ppm), enabled quantitative analysis of microplastics with a lower limit of detection. Microplastic concentrations were determined by measuring the intensity of the characteristic peak of RhB. We then validated our method by detecting microplastics in tap water.
Results: The Zr^{4+} assisted RhB labeling strategy enabled the binding of RhB with microplastics, as validated by Confocal Laser Scanning Microscopy. By coupling this labeling strategy with SERS measurement, we achieved accurate detection of polystyrene at concentrations as low as 0.1 ppm, with a detection limit of 1 ppb. Our method also generated around 10-fold higher SERS intensity compared to direct SERS measurement for microplastics. Additionally, our research established a linear relationship between RhB SERS intensity (Y) and polystyrene concentration (X) for quantitative analysis, represented by the equation $Y = 179.5X + 248.3$. The application of our method in real-world scenarios, such as analyzing microplastics in tap water, demonstrated high trapping effectiveness. The accuracy of our method achieved over 90% for measuring the microplastic concentration range of 5 ppm to 30 ppm.
Significance: This innovative approach offers an useful tool for the on-site, sensitive, and cost-effective detection of microplastics. With our method, individuals can easily and accurately measure the presence of microplastics and monitor the microplastic concentration in the environment using portable Raman equipment, thereby ensuring agri-food safety.

P1-264 Accurate Classification of Nanoplastics following Metal Phenolic Networks-Mediated Separation via Machine Learning Aided SERS Detection

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❖ ***Developing Scientist Entrant***

Introduction: The increasing accumulation of nanoplastics across agri-food systems holds significant threats to both terrestrial and aquatic life but their identification
Purpose: for sensitive detection and accurate classification of nanoplastics through metal phenolic networks (MPNs)-mediated separation.
Methods: MPNs are served as rapid concentration and separation of various types and sizes of nanoplastics (50 nm PS, 500 nm PS, 500 nm PMMA, 250 nm PLA) followed by SERS detection. We established a considering the complete region of characteristic peaks across diverse nanoplastics of SERS spectra instead of traditional manual analysis on a singular characteristic peak. We then validated the developed method for nanoplastics detection in real-world scenarios.

Results: MPNs is an effective strategy for separation of 500 nm polystyrene as validated by Transmission Electron Microscopy (TEM) images. By coupling MPNs-mediated separation with SERS and tailored machine learning strategies, we can achieve sensitive detection (down to 0.1 ppm) and accurate classification (97%) of various types of nanoplastics. The effectiveness and novelty of this approach is substantiated by its ability to discern different nanoplastics mixtures and detect nanoplastics samples in both tap and lake water systems.

Significance: Our method offers cost-effective, rapid, field-deployable detection features with excellent sensitivity in nanoplastics analysis. It will serve as a promising means to ensure long-term food safety and promote sustained food protection.

P1-265 Identifying Food Adulteration: Reliable and Sensitive Detection of Contaminants in Halal Foods, Camel Milk, and Coffee Beans

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Introduction: Current methods for food adulteration testing often lack efficiency, cost-effectiveness, and/or regulatory alignment, leading to economic repercussions, diminished consumer confidence, and compliance uncertainties. This study uses the Maxwell® RSC PureFood GMO and Authentication Kit with downstream qPCR or SNP analysis for sensitive detection of contaminating DNA in diverse food products, addressing these challenges.

Purpose: We evaluated the ability of these systems to accurately detect DNA contamination in various food matrices, aiming to reinforce consumer trust and align with ethical considerations important in the food safety market.

Methods: Commercially available Halal and non-Halal products (ground beef, pork, chicken nuggets, hot dogs, etc.), powdered camel milk, and Arabica and Robusta coffees were tested. Samples (100mg-200mg) were spiked with respective contaminating products (0.01%-100%, by mass). DNA was purified from spiked or pure samples (n=3-4) and was used for subsequent qPCR or SNP analysis to detect contaminating DNA. Amplification linearity (R^2≥0.98=-pass), efficiency (>90%=pass), and limit of detection (≤0.1%=pass) were assessed. Sequence electropherograms were analyzed to ensure adequate coverage and visualization of the loci of interest.

Results: DNA purification was successful for all samples. Specific detection of non-Halal DNA was achieved in 161/168 samples of varying type and concentration (LODs ≥0.01% for ground meats and ≥0.1% for other matrices), and all metrics passed for all sample types, except one (powdered soup stock). Detection of powdered camel milk adulteration with ≥1% powdered cow milk was achieved in 42/42 samples of varying contamination percentage, and all metrics passed. Analysis of two SNP loci allowed for successful detection of ≥10% Robusta contamination in Arabica coffee with acceptable sequencing quality metrics.

Significance: This study demonstrates sensitive and reliable approaches for detecting adulteration in diverse food products, addressing the technical demands of food identity verification and the broader economic, market, and regulatory needs of the evolving food industry.

P1-266 The GENE-UP® PRO™ Animal Species ID Method Enables Rapid Identification and Contamination Detection of Animal Species in Meat Samples

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Introduction: Fraudulent meat adulteration is of concern to the food industry, as undeclared animal species in meat products can affect consumers' rights and compliance with food labeling requirements. The GENE-UP® PRO™ Animal Species ID is a novel DNA-based solution for meat testing. This method is comprised of thirteen individual TaqMan™ real-time PCR assays targeting species of bovine, equine, elk, deer, pork, lamb, goat, chicken, turkey, duck, rabbit, cat and dog.

Purpose: To evaluate the ability of the PCR method to identify and detect low-level contamination of animal species from meat tissue samples.

Methods: Authenticated meat samples were collected from three or more different specimens of the thirteen animal groups (n=54). Isolation of DNA from tissue was carried out using a commercial DNA purification kit. Following, DNA was quantified and normalized to a range of 5 to 10 ng/µL. The specificity and sensitivity of the method were evaluated. The PCR assays were tested with three replicates of normalized DNA from samples of all animal groups, as well as serial dilutions of target DNA to establish the limit of detection (LOD). The ability to detect meat adulteration was evaluated by testing limiting concentrations of contaminating DNA in sample mixes of closely related animal species.

Results: The results showed good specificity, as no cross-amplification of non-target species was observed at a DNA concentration range of 5 to 10 ng/µL. The LOD of this method was 20 pg/µL of purified DNA on all thirteen animal species assays. The method was able to accurately discriminate target DNA at a concentration of at least 1% from a mixed sample of genetically similar species.

Significance: The animal species real-time PCR is a rapid and robust method for the identification and detection of low-level cross-contamination of animal species in meat samples.

P1-267 Comparing the Performance of Species Identification in Bovine and Swine Origin Meat Samples Between ID-Check Kits and a Fourplex Identification Kit

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Introduction: Identifying meat species is essential to food safety, particularly for transparency in labeling and in reporting of origins and economic transactions. Due to meat-adulteration scandals in the international food supply chain, identifying analytical tools with high specificity and sensitivity for detection of meat species is significant.

Purpose: In this study, the specificity and sensitivity of the Bio-Rad ID-Check Bovine and Swine Kits were validated, and the performance comparison on each target was conducted against a competitor's fourplex kit.

Methods: For evaluating the specificity, 20 samples consisting of food ingredients that were negative of the targets were analyzed using ID-Check Kits to check for cross-reactivity. For validating the sensitivity of ID-Check Kits and comparing it with the fourplex kit, two levels of target DNA (0.001% and 0.01%) were spiked in samples (n=20). A Paired T-Test for statistical difference was performed at 95% confidence.

Results: For the specificity evaluation, no cross reaction was observed in the 20 samples free of targets for both ID-Check Bovine and Swine Kits. For the sensitivity evaluation, the results obtained by using the ID-Check Bovine and Swine Kits achieve 100% agreement with all samples spiked at 0.01% and 0.001%. In comparison to the fourplex kits, Cq from the ID-Check Kits for bovine and swine at both spiked levels were statistically lower than the fourplex kits (p<0.0001). In evaluating the performance of the bovine target, Cq from ID-Check Kit was on average 4.378 and 3.578 lower than values from the fourplex kit at spike level 0.01% and 0.1%, respectively. In evaluating the performance of the swine target, the Cq obtained from ID-Check Kit was on average 5.942 and 6.944 lower than values from the fourplex kit at spike level 0.01% and 0.1%, respectively.

Significance: Results obtained from this study indicated that Bio Rad ID-Check Bovine and Swine Kits was highly specific and sensitive with greater sensitivity compared to a competitor's fourplex kits.

P1-268 Beyond qPCR: Harnessing the Power of Droplet Digital PCR (ddPCR) to Assess Concentrations and Pathogenicity of Shiga toxin-producing *E. coli* (STEC) in Agricultural Irrigation Water

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Introduction: Testing agricultural irrigation water is advantageous since it has been implicated in Shiga toxin-producing *Escherichia coli* (STEC)-produce outbreaks. Authenticating STEC concentrations in agricultural waters could improve risk assessments. The detection limit of qPCR (103 – 104 CFU/mL) is such that low STEC concentrations could be missed in unenriched samples, especially when originating from large water volumes. Droplet digital PCR (ddPCR) can provide absolute quantification, with a detection limit of 102 CFU/mL and is less prone to PCR inhibition.

Methods: 10L, 50L, and 100L of agricultural water was collected in duplicate by dead-end ultrafiltration (DEUF). Filters were spiked with approximately 10^2 or 10^4 CFU of *E. coli* O157:H7. Backflush from filters was tested with and without enrichment with qPCR (FDA BAM Chapter 4a) and ddPCR (Bio-Rad; ddCheck STEC kit). Detection enhancements, using additional microfiltration and concentration steps, were examined.

Results: Equivocal detection from enriched samples was observed using both qPCR and ddPCR methods. Concentrated, unenriched backflush, with 4.5 CFU/µL enterohemorrhagic *E. coli* (EHEC) O157:H7, was subjected to qPCR and ddPCR to quantify contamination levels in the original source water. qPCR yielded Ct values (>32.0) for 3 out of 6 samples, but ddPCR had positive droplets detected in all samples. Approximately 400mLs of backflush from enriched and unenriched 10L filtered samples was subjected to a novel microfiltration and concentration step, thus reducing sample volume approximately 200-fold. ddPCR results yielded positive droplets ranging from 17.24 to 29.63 and linkage analysis (*stx* and *eae*) provided confirmation of EHEC. qPCR Ct values for these samples were in the mid-30s.

Significance: Results demonstrate that ddPCR can potentially quantify EHEC *and STEC* with high precision and accuracy. Robust detection in unenriched samples is possible with additional microfiltration and concentration steps, thus removing a barrier to quantifying the original contamination level.

P1-269 Validation of an Alternative Method for Shiga-Toxin-Producing *Escherichia coli* (STEC) Detection in Broad Range of Food

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Introduction: An analysis of recent Shiga-toxin-producing *Escherichia coli* (STEC) outbreak in EU and US showed a spread of this contaminant across more and more diverse food products. The need for novel methods capable of analyzing a broad range of food items is essential for effective surveillance and prompt response to emerging outbreaks.

Purpose: For the first time, an independent study evaluates the performance of an alternative method for the detection of STEC in a broad range of food products against the ISO/TS 13136:2012 reference method and following ISO 16140-2:2016 standard.

Methods: The alternative method is a real-time PCR assay (iQ-Check VirX, Bio-Rad) targeting *stx* virulence genes. After an enrichment at 41.5±1°C in Buffered Peptone Water supplemented or not (STEC supplement, Bio-Rad), DNA is extracted and screened by PCR. The alternative method was compared to ISO/TS 13136:2012 to evaluate its sensitivity and relative level of detection (RLOD). Five food categories were evaluated: raw dairy products, raw meat products, flours and raw doughs, multicomponent foods, raw produces and fruits.

Results: Overall, 319 samples were analyzed for the sensitivity study, providing 151 *stx* confirmed positives with the alternative method and 118 confirmed positives with the reference method. Among those positives 16 samples were naturally contaminated. Depending on the protocol evaluated, the sensitivity of the alternative method ranged between 83.2% and 89.3% while the reference method ranged between 69.8% and 71.1%. RLOD, varying between 0.276 to 1.321 depending on the matrix tested, suggests similar detection capabilities for both methods.

Significance: The study highlights comparable or improved performances of the alternative method leading to a broad range of food certification by MicroVal Technical Committee.

P1-270 Simultaneous Dual-Gene Detection of *Escherichia coli* O157:H7 Based on CRISPR/Cas13-Mediated Biosensor

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❖ ***Developing Scientist Entrant***

Introduction: Shiga toxin-producing *Escherichia coli* O157:H7 (*E. coli* O157:H7) causes a large number of foodborne outbreaks worldwide each year.

Purpose: Due to the high genomic similarity of different E. coli serotypes, it is difficult to distinguish *E. coli* O157:H7 from other *E. coli*. To address this issue, we used bioinformatic analysis to locate specific genes encoding *E. coli* O antigen and H antigen, then developed a CRISPR/Cas13-mediated biosensor for simultaneous dual-gene detection of *E. coli* O157:H7.

Methods: Two Cas13 nucleases (LwaCas13a, PsmCas13b) were utilized to target the $rfbE_{O157}$ and $fliC_{H7}$ genes in *E. coli*, respectively. In the presence of *E. coli* O157:H7, Cas13 nucleases can recognize $rfbE_{O157}$ and $fliC_{H7}$ gene simultaneously, triggering unique dinucleotide preferences for two fluorescent probes by *trans*-cleavage, which in turn generates a dual-channel fluorescent signal.

Results: Integrated with recombinase polymerase amplification (RPA), the proposed CRISPR/Cas13-mediated biosensor successfully distinguished *E. coli* O157:H7 from other *E. coli* serotypes. Impressively, this biosensor has a detection limit as low as 87 CFU/mL for *E. coli* O157:H7, and 100% accuracy in milk samples.

Significance: This biosensor provides a more accurate and convenient pathway to identify *E. coli* O157:H7 in the food supply chain.

P1-271 Recovery and Detection of Shiga-Toxin Producing *Escherichia coli* and *Salmonella* spp. from Polyolefin Cloth (Microtally®) Using Non-Proprietary Media and a Loop-Mediated Isothermal DNA Amplification Method

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Introduction: In January of 2023, the United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS) issued a notice describing the use of a non-destructive surface sample collection method to collect raw beef manufacturing trimming and bench trim verification samples equivalent to the N60 excision sampling. Thus, it is important to assess the recovery and detection of foodborne pathogens from cloth material before a method is implemented for routine use in testing laboratories.

Objective: To evaluate dual detection of low levels Shiga-Toxin Producing *E. coli* (STEC) and *Salmonella* from polyolefin cloth (Microtally) utilizing buffer peptone water ISO (BPW-ISO) and loop-mediated isothermal DNA amplification (LAMP).

Methods: Beef trim was obtained from local supermarkets and placed into a tray (~10Kg) to mimic the sample collection process. The cloths (N=60) were used to massage the surface of the beef trim as described by FSIS (Notice 05-23). After massaging cloths were artificially spiked with a theoretical amount of 1 CFU of *E. coli* O157:H7 (stx+/eae+) *S. enterica* sv Typhimurium (ATCC14028) and refrigerated 48h for conditioning. Thirty swabs were enriched with 200 of BPW-ISO at 41.5oC and testing aliquots were analyzed after 8, 10, 12 and 24h. Additionally, thirty swabs were enriched with 200mL of modified tryptic soy broth at 41.5oC for 24 h. All samples were cultured confirmed.

Poster

Results: The relative limit of detection was calculated for the two enrichment schemes following AOAC guidelines, the p-value when comparing molecular screening and culture confirmation of *E. coli* O157, STEC and *Salmonella* was >0.05. The shortest enrichment (8h) evaluated, demonstrated to be sufficient to detect and recover both microorganisms from Microtally® cloths using BPW-ISO and LAMP.

Significance: Implementation of an enrichment scheme with non-proprietary media and LAMP detection offer testing laboratories a fast method to analyze raw beef and bench trim verification samples.

P1-272 A Novel Study Design to Compare Manual and Automated PCR-setup Workflows

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Introduction: The Thermo Scientific™ SureTect™ Automation Platform offers a streamlined automated workflow for the Thermo Scientific™ SureTect™ assays to enable detection and differentiation of the most common food pathogens from various food and environmental samples.

Purpose: A study design was developed to provide a robust comparison of the two workflows to validate the performance equivalency of the automated and manual methods.

Methods: A paired comparison study design included enrichment and post-enrichment inoculation to the required contamination level of selected food matrices known to be challenging to lysis and PCR. Serial dilution of the inoculated aliquot was prepared using enriched test portion broth to enable testing above LOD, at LOD, and below LOD. Dilutions were run through the automated and manual workflows (16 replicates each) and analyzed by qPCR using Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument. Results analysis included difference in C_t averages for dilutions above LOD, number of positives per dilution, and statistical evaluation of number of positives using the Wilrich and Wilrich statistical calculator and the AOAC POD calculator.

Results: Comparison studies above the LOD of the PCR assays showed that the difference in average Ct values were always within ±1.5 cycles and 90% confidence intervals when comparing the automated and manual procedures. The LOD for the automated and manual procedures was compared using the Wilrich and Wilrich statistical calculator and the AOAC Probability of Detection (POD) calculator. At the LOD, the numbers of positives per dilution for each assay-matrix combination was statistically comparable when comparing the automated procedure to the manual and acceptability limits for LOD_{50} results were achieved, demonstrating that the two workflows are statistically comparable in performance.

Significance: The study design provided a robust and accurate assessment of the two workflows and could be employed in future studies where similar method changes are made.

P1-273 Detection, Quantitation, and Serotyping of *Salmonella* from Poultry Samples with Harmonized Protocols

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*Intro***:** Salmonella contamination in poultry can pose significant health risks to consumers, therefore rigorous testing protocols are required to ensure safe consumption. Thermo Fisher Scientific offers a range of detection, quantitation, and serotyping PCR methods for fast and accurate detection and enumeration of *Salmonella* species and specific serotypes in poultry samples.

Purpose: Harmonize test portion dilution and enrichment for raw poultry products and poultry rinses using the Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit (quantitation), Thermo Scientific™ SureTect™ Salmonella species PCR Assay (detection), Thermo Scientific™ RapidFinder™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit, and Thermo Scientific™ SureTect™ Salmonella Infantis PCR Assay (detection and serotyping) methods.

Method: Matrix studies were conducted according to AOAC *Appendix J* guidelines comparing the performance of the Thermo Scientific PCR methods to the USDA FSIS MLG 4.14, Isolation and Identification of Salmonella reference method. For each matrix, a harmonized test portion dilution and enrichment protocol was followed across all Thermo Scientific PCR methods.

Results: For quantitation, the SureCount method had a statistically comparable performance to the reference method and met AOAC performance requirements. For detection, the SureTect Salmonella species PCR Assay, RapidFinder Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit, and SureTect Salmonella Infantis PCR Assay methods all had a statistically comparable performance to the reference method and met AOAC performance requirements.

Significance: The Thermo Scientific PCR end-to-end workflow for *Salmonella* testing in poultry offers end users the ability to detect and quantitate *Salmonella* species as well as differentiate specific serotypes from a single test portion, accurately and reliably.

P1-274 *Salmonella* Species Detection in Pet Food Using a Well-Established RT-PCR Method

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Introduction: *Salmonella* is a major global foodborne pathogen with a severe impact to public health. The Thermo Scientific™ SureTect™ Salmonella species PCR Assay provides an accurate and reliable method for the detection of *Salmonella* from a broad range of foods, environmental samples, primary production samples, pet foods and animal feeds. The SureTect Salmonella PCR method holds a number of certifications, including ISO 16140-2:2016 (AFNOR and MicroVal), and AOAC PTM and OMA.

Purpose: Demonstrate the accuracy and reliability of the SureTect Salmonella PCR method for the detection of *Salmonella* in large sample sizes of pet food.

Method: Probability of Detection (POD) matrix studies were conducted according to AOAC Appendix J on 25g dry dog food, 25g wet cat food, up to 375g cat liver pâté, and up to 150 g of cat kibble. The SureTect method was compared to the ISO 6579-1:2017 reference method. For each matrix, 5 blank portions, 20 low level contamination portions, and 5 high level contamination portions were tested by both methods following an unpaired study design.

Results: The difference in POD (dPOD) of the two methods at the fractional inoculation level was 0.15 for 25 g dry dog food, 0.00 for 25 g wet cat food, 0.15 for 375 g cat liver pâté, and -0.05 for 150 g cat kibble. The positive dPOD values indicate a stronger performance by the SureTect Salmonella PCR method versus the reference method. The results demonstrate there is no statistically significant difference between the performance of the methods.

Conclusion: The SureTect Salmonella species PCR Assay was proven to be an accurate and reliable method for the detection of *Salmonella* from a variety of challenging-to-test pet foods with a test portion up to 375g.

P1-275 Harmonized Large Sample size (375 g) Testing for Detection of *Salmonella* and *Cronobacter* spp. in Powdered Infant Formula Using Thermo Scientific SureTect *Salmonella* PCR Assay and Thermo Scientific SureTect *Cronobacter* PCR Assay Granted AOAC PTM

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Introduction: Annually, *Salmonella* and *Cronobacter* are responsible for over 200 million infections worldwide, with an outbreak of *Cronobacter* occurring in the US in 2022. One such source of infection is powdered infant formula (PIF), which is especially concerning for patients with suppressed immunity such as neonates, with case mortality reported to be 50-80%. Sensitive, easy, and reliable methods to detect *Salmonella* and *Cronobacter* spp. in PIF are of fundamental importance to reduce global disease burden.

Purpose: To evaluate for AOAC PTM certification the Thermo Scientific™ SureTect™ Salmonella species PCR Assay and Thermo Scientific™ SureTect™ Cronobacter species Assay harmonized enrichment for large PIF sample sizes and environmental samples to detect *Salmonella* and *Cronobacter* spp. The harmonized enrichment involves a 1-in-6 dilution of sample of Buffered Peptone Water (with the addition of 6mg/L of Novobiocin for probiotic samples) with both assays run from the same lysate.

Methods: The validation study adhered to AOAC Appendix J guidelines following an unpaired study design. The probability of detection (POD) study included 30 samples at three different contamination levels. Inclusivity/exclusivity studies consisted of 50 target isolates and 30 non-target isolates. Product stability and robustness was also conducted.

Results: POD analysis showed either no statistically significant differences between methods or superior performance in favor of the SureTect methods. All inclusivity/exclusivity isolates were successfully detected and excluded respectively, and robustness and stability studies showed no performance deviations.

Significance: The harmonized enrichment allows for rapid and accurate dual detection of *Salmonella* and *Cronobacter* spp. from large sample sizes of PIF and environmental samples. The enrichment is simple, with a low dilution ratio and antibiotic addition required only for probiotic PIF. AOAC PTM approval was granted following previous ISO 16140-2 approval.

P1-276 Detection of *Campylobacter* from Raw Milk and Raw Pork Using a Multiplex PCR Workflow

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Introduction: *Campylobacter* is the leading cause of gastroenteritis disease worldwide, is commonly associated with poultry products, and has been associated with outbreaks from raw milk and raw pork. The Thermo Scientific™ SureTect™ *Campylobacter jejuni*, *C. coli* and *C. lari* PCR Kit is used for the detection of three species from poultry matrices. It is proposed to verify the performance of the PCR workflow for the detection of *Campylobacter* from raw milk and raw pork.

Purpose: The purpose of the study was to verify the performance of the SureTect molecular workflow for the detection of *Campylobacter* from raw milk and raw pork matrices.

Methods: Raw milk (n=18) and pork matrices (n=24) were artificially contaminated with target *Campylobacter* at 1-38 CFU per 25 g sample. All samples were enriched in pre-warmed Bolton Broth without blood, in enrichment bags with minimal air headspace and incubated at 42°C for 22-30 hours. Samples were tested with the molecular workflow and streaked to culture media for confirmation.

Results: The molecular workflow successfully recovered and detected all artificially contaminated strains from raw milk and raw pork matrices after 22 hours enrichment.

Significance: The data demonstrated that the molecular workflow is able to reliably detect *Campylobacter* from raw milk and raw pork matrices, providing confidence that the products are safe to consume.

P1-277 Harmonized AOAC PTM PCR Detection Workflows for *Salmonella* and STEC from 375 g Raw Beef and Leafy Produce Samples

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Introduction: *Salmonella* and Shiga-toxin producing *Escherichia coli* are important food pathogens in raw beef and leafy produce. A harmonized PCR workflow has been developed to enable detection of both pathogens from one short enrichment protocol using a large 375 g sample. Providing end users with a reliable, fast and streamlined method.

Objective: Develop and analyze a harmonized protocol for 375 g raw beef trim and leafy produce samples using the Thermo Scientific™ Salmonella species PCR Assay and the Thermo Scientific™ SureTect™ Escherichia coli O157:H7 and STEC Screening PCR Assay with the Thermo Scientific™ SureTect™ Escherichia coli STEC Identification PCR Assay.

Method: Studies for 375g raw beef trim (1-in-5 pre-warmed buffered peptone water (BPW), 41.5°C, 8-24 hours) and 375g raw leafy produce (1-in-10 pre-warmed BPW, 41.5°C, 10-24 hours) were conducted and analyzed according to the AOAC Appendix J. The SureTect Salmonella method was compared to the ISO 6579-1:2017 and FSIS MLG Ch.5 methods, and the SureTect STEC method was compared to the ISO/TS 13136:2012 method.

Results: The SureTect methods showed superior or comparable performance to the corresponding reference methods with no statistically significant differences. The 375 g raw beef trim had a difference in probability of detection value between the SureTect and reference method (dPOD) of 0.05 for the Salmonella method and 0.10 for the STEC method. The 375 g leafy produce had a dPOD of -0.05 for the Salmonella method and 0.10 for the STEC method. The harmonized SureTect workflows as described have been successfully certified by AOAC PTM.

Significance: Harmonized detection of two pathogens *Salmonella* and STEC from one large sample size enrichment, and therefore a single lysate, provides multiple aspects of efficiency for end users. Additionally, the 1-in-5 enrichment ratio for large beef samples also saves on media usage and space, and therefore cost and efficiency.

P1-278 Detection of Shiga Toxin-Producing *Escherichia coli* in Raw Flour-Based Foods

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) outbreaks are typically associated with raw meat products and fresh produce. Flour has recently been a source of human illness caused by STEC. In 2018, a multi-state outbreak caused by contaminated flour resulted in three hospitalizations out of 21 reported cases. A survivability study assessing STEC contamination of flour found that 20% samples tested positive for the *stx* gene after two years' storage. Screening of flour products for STEC may be used in effective risk mitigation strategies.

Purpose: To verify performance of the Thermo Scientific™ SureTect™ molecular workflow versus a culture media method for detection of STEC in flour-based foods.

Methods: A verification study compared the results of the molecular method against the culture media method. Twenty flour-based products and twen-

ty STEC isolates were tested in a paired study. Samples were enriched at 37°C for 18 hours with the addition of alpha amylase, in accordance with ISO 6887 for high starch matrices. The first study tested ten flour-based products. STEC isolates were artificially contaminated at a low level (1.2 – 9 CFU/sample) before ambient storage for one-week prior testing. A second study was removed the storage step, due high levels of die-off observed during the first study.

Results: The molecular workflow identified 23 positive samples, while the culture media method identified 25. A chi-squared test concluded that there was no significant difference between the two methods, X^2 (19, n=80) = 17.23, p=0.05. The difference may be attributed to relative sensitivity between techniques and the need for multiple gene targets to generate a positive result with the molecular workflow; specific matrices may require extended enrichment to account for this.

Significance: The molecular workflow was comparable to the culture media workflow and gave presumptive results >18 hours earlier, allowing for faster decision-making during manufacturing.

P1-279 Evaluation of Multiple Harmonized PCR Workflows for Detection of Bacteria from Infant Formula

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Introduction: The rapid and reliable detection of bacteria from liquid infant formula is vital to ensure these products are safe to consume, and product can be released to the market rapidly. Thermo Scientific™ SureTect™ rapid PCR workflows for a range of pathogens can be used to test these products including *Salmonella, Cronobacter,* and *S. aureus*.

Purpose: This study evaluated the performance of five different PCR Assays for the detection of bacteria from liquid infant formula and demonstrates that a harmonised enrichment and lysis protocol can be used for those workflows, saving cost and time to the end user.

Methods: Forty-eight samples of liquid infant formula were tested, including both artificially contaminated and non-contaminated samples. Four strains of different bacteria were used to artificially contaminate samples at a level of <10 CFU per sample. Enrichments were prepared in Buffered Peptone Water (harmonized for *Salmonella*, *E. coli* and *Cronobacter* testing) or Giolitti-Cantoni Broth (*S. aureus* testing). All samples were processed with a harmonized lysis step, prior to testing with PCR. The performance of the PCR workflows was evaluated by comparing results to culture confirmation methods.

Results: All PCR workflows were able to detect the target strains with 100% of results matching culture confirmation results. Where thermal cycler parameters were updated to provide a harmonized workflow for *E. coli* detection, performance was maintained appropriately.

Significance: The PCR workflows reliably detected each target from liquid infant formula using harmonized enrichment and lysis protocols which reduces cost and total effort in the laboratory.

P1-280 Use of Visible Dye to Prevent Supplementation Error in Rapid Workflows for Detection of *Salmonella* and *Cronobacter*

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Introduction: Novobiocin supplements included for use with the Thermo Scientific™ SureTect™ and Precis™ *Salmonella* and *Cronobacter* workflows are colorless and challenging to track when added to sample enrichments. The inclusion of an inert dye to the antibiotic benefits laboratory staff by providing a visible indication that the antibiotic has been added to the media. This approach improves supplementation tracking and prevents mistakes in sample preparation.

Purpose: To evaluate the performance of a liquid format of novobiocin that includes a visible dye (alternative supplement) for inclusion with proprietary enrichment methods associated with the Precis and SureTect methods for *Cronobacter* spp. and *Salmonella* spp. detection.

Methods: The alternative supplement was compared to a standard novobiocin supplement without dye using an unpaired study design. The food matrix sensitivity study adhered to AFNOR spiking and ISO 161410-2 rules but analyzed a smaller sample size. Five food categories were tested for *Salmonella* (n=87) and one for *Cronobacter* (n=15). The relative limit of detection (RLOD) study contained three categories (25 g ground beef and 375 g pet food for *Salmonella,* and 375 g powdered infant formula for *Cronobacter)* with 20 fractional and 5 high-spiked samples per category. The inclusivity and exclusivity studies for each pathogen analyzed >50 and >10 isolates, respectively.

Results: The alternative supplement demonstrated comparable performance to the standard supplement. The *Salmonella* sensitivity study returned 12 positive deviations and 7 negative deviations, whilst the *Cronobacter* sensitivity study returned 2 positive deviations and 1 negative deviation. The RLOD for both *Salmonella* and *Cronobacter* were below the acceptability limit of 2.5. All *Cronobacter* and *Salmonella* inclusivity isolates were successfully identified, and all exclusivity isolates were successfully excluded.

Significance: Liquid novobiocin with dye performs comparably to antibiotic without dye, providing a reliable and easily visible indicator which improves sample supplementation tracking and reduces testing errors.

P1-281 Detection of Only Live *Escherichia coli* with Long-Read Sequencing

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Introduction: Long-read sequencing shows promise as a method for detecting foodborne pathogens, but it cannot differentiate between live and dead bacteria.

Purpose: The objective of this study was to evaluate the use of propidium monoazide (PMA) to prevent the sequencing of DNA from dead *Escherichia coli* O157:H7.

Methods: Two independent experiments were performed each with the following treatments prepared in triplicate using 10^8 CFU/mL *E. coli*: live bacteria with no PMA treatment, live bacteria treated with 25 µM PMA, dead bacteria with no PMA treatment, and dead bacteria with 25 µM PMA. The dead bacteria had been heat killed for 4 minutes at 90 °C. Samples were plated to confirm the concentration of the live and dead samples. DNA extractions from each sample were tested using qPCR and sequenced on an Oxford Nanopore Technologies MinION Mk1b to determine if the genes *fliC*, *eae*, *stx1*, *stx2*, and *rrsC* could be detected.

Results: Results were analyzed with a t test and there were no significant differences between detection of the virulence genes in the PMA-treated live *E. coli,* the non-treated live control, or the non-treated dead control in either the qPCR or sequencing assays. However, none of the virulence genes were detected in dead PMA-treated *E. coli* in the qPCR or sequencing assays as compared to live PMA-treated *E. coli*, and this result was significant (p<0.0001).

Significance: Long-read sequencing could be a faster, less labor-intensive, and more cost-effective method to detect foodborne pathogens. The ability to differentiate between live and dead bacteria using PMA treatment prior to sequencing will remedy one of the main disadvantages of sequencing for pathogen detection.

P1-282 Validation of the Bio-Rad dd-Check STEC Solution for the Detection and Analytical Confirmation of Shiga Toxin-Producing *Escherichia coli* in Raw Beef Trim, Fresh Spinach and Carcass Sampling Cloths

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Introduction: Current qPCR methods for screening STEC have common challenges of separating enrichments with linked and unlinked virulence. Bio-Rad's dd-check STEC Solution uses ddPCR technology which allows differentiation of true-positive samples with coexistence of Shiga toxin (stx 1/2) and intimin (eae) genes within a single bacterium from false-positive samples in which virulence genes originate from different bacteria.
Purpose: A validation study was performed to evaluate the candidate method as a screening assay for detection of STEC in raw beef trim (325g and 375g), fresh spinach (200g and 375g) and carcass sampling cloths. The study also examined the candidate method's performance in STEC analytical confirmation with four different fresh and frozen enrichments.
Results: In the inclusivity and exclusivity study, all 100 STEC isolates were detected, and all 100 exclusivity organisms were not detected. The robustness, product consistency and stability, and instrument variation studies demonstrated no statistically significant differences. There were no statistical differences detected between presumptive and confirmed results or between candidate and reference methods results for either the screening or analytical confirmation protocols at all time points. The results obtained using sample enrichments that were frozen up to 7 days showed no statistical significance compared to using fresh sample enrichments. In conclusion, the data from these studies support the the candidate method as an applicable tool for detection and confirmation of STEC in raw beef trim, fresh spinach, and carcass sampling cloths.
Significance: The the candidate method is an accurate method for detecting and confirming true STEC positive samples. This detection and linkage verification of targets in a single bacterium enhances the accuracy of food testing by reducing the number of false-positive results. Using the the candidate method following a presumptive PCR positive STEC result has proven to be an effective cultural-independent confirmation tool for true STEC positive samples in less than 24 h.

P1-283 Approach of a Molecular Biological Diagnostic Pretreatment Utilizing Functionalized Magnetic Nanoparticles for Rapid Detection of *Escherichia coli* O157:H7

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✦ ***Undergraduate Student Award Entrant***

Introduction: Molecular biological diagnostics such as real-time PCR, commonly proposed for the rapid detection of foodborne pathogens, necessitate efficient DNA extraction process.
Purpose: This study was to develop pretreatment methods for *Escherichia coli* O157:H7 combining pathogen concentration and DNA extraction for molecular diagnostic using two types of functionalized magnetic nanoparticles (MNPs).
Methods: Amine compound-coated MNPs (AMNPs) and silica-coated MNPs (SMNPs) were utilized for pathogen magnetic concentration and DNA magnetic isolation, respectively. To optimize the capture efficiency of *E. coli* O157:H7 by AMNPs, we tested three types of buffers, amounts of AMNPs from 10 to 140 µg/mL, and incubation times from 1 to 20 minute. Furthermore, three combinations utilizing AMNPs and SMNPs were performed and assessed in comparison to original protocol without AMNPs. To validate this optimized method, it was compared with pretreatment methods using centrifuge and boiling. DNA extracted from culture of 10^0 to 10^9 CFU/mL was detected through real-time PCR and measured through nanodrop analysis.
Results: AMNPs demonstrated a significant capture efficiency of 98.71% with amount of 80 µg/mL during 5 minutes of incubation with *E. coli* O157:H7 in distilled water. Among the three tested combinations, the approach involving the isolation of AMNPs after the lysis process and subsequent addition of SMNPs exhibited no statistically significant difference from the original protocol without AMNPs. Comparative analysis revealed that this MNPs method exhibited the highest purity (260/230) with a value of 2.00 ± 0.11 (p<0.001) and maintained an equivalent detection limit of 10^3 CFU/mL compared to other pretreatment methods.
Significance: This developed method offers the possibility of simple automation using MNPs and magnets, representing potential for rapid and sensitive detection of *E. coli* O157:H7 as well as other foodborne pathogens.

P1-284 Verification of Several Dietary Supplements and Nutraceuticals Using the GENE-UP® *Salmonella* (SLM), *Listeria spp.* (LIS) and, *Listeria monocytogenes* (LMO) Assays

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Introduction: Molecular and microbiological pathogenic testing in dietary supplements and nutraceuticals is important for human health. However, these products can pose challenges to pathogen testing due to their complex inherent natural properties. Therefore, it is vital to have reliable, rapid tests and streamlined workflows to meet the demand of safe products in the market.
Purpose: The performance of real-time (RT) PCR assays that detect *Salmonella*, *Listeria* spp. and *Listeria monocytogenes* was verified in several dietary supplements and nutraceutical products and confirmed by FDA BAM Chapter 5 and 10 culture methods.
Methods: A total of 20 dietary supplements and nutraceutical products were evaluated each consisting of 7 inoculated replicates and 1 negative control of 25g matrices. Two sets of matrices for each product were each inoculated with *Salmonella* spp. and *L. monocytogenes* at 3-14 CFU and enriched in BPW for *Salmonella* and LPT for *Listeria* at various dilution ranges from 1:10 - 1:50 for optimal pathogenic growth, recovery, and detection. Two out of 20 matrices were tested at 1g since growth and recovery of pathogens required a 1:1000 enrichment. One of these matrices required secondary enrichment for *Salmonella* growth and recovery. *Salmonella* sets incubated at 42 °C for 18-24h and *Listeria* sets incubated at 35 °C for 22-24h were run through RT-PCR following GENE-UP SLM, LIS and LMO package inserts. Alternative and reference confirmations were performed on every sample regardless of screening result.
Results: Results indicated positive detection within acceptable PCR ranges for pathogen targets for all matrices screened and 100% alignment with both alternate and reference confirmations. All uninoculated controls tested negative for target assays and reference confirmations.
Significance: These data indicate that the SLM, LMO and LIS assays are suitable for detecting *Salmonella* and *L. monocytogenes* pathogens from challenging dietary supplements and nutraceuticals.

P1-285 Molecular Detection of *Salmonella* and *Listeria* in Food Flavorings Using GENE-UP® LIS, LMO and SLM

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Introduction: Flavorings used for enhancing the mouthfeel experience of foods can be a source of pathogenic contamination. Their inherent properties can also pose challenges to detecting food pathogens using molecular detection tests.
Purpose: The performance of real-time (RT) PCR assays were evaluated in detecting *Salmonella* and *Listeria* in several food flavoring matrices.
Methods: Four food flavorings were evaluated using AOAC style validation studies. Two 375g matrices were inoculated with *Salmonella* spp. and two 25g matrices were inoculated with *L. monocytogenes* strains. Each study consisted of 20 replicates inoculated with target strains at fractional levels (~0.0-2.0 CFU), 5 replicates at high levels (~5.0 CFU) and 5 replicates at uninoculated levels. *Salmonella* inoculated samples were enriched at 1:20 or 1:30 with a

secondary regrow and incubated overnight at 35°C. *Listeria* inoculated samples were enriched at 1:10 and 1:30 and incubated overnight at 35°C. Samples were evaluated on GENE-UP SLM2, LIS and LMO assays and confirmed to alternative and traditional FDA BAM methods; Chapter 5 for *Salmonella* and Chapter 10 for *Listeria*. Reference sets were also run simultaneously with the candidate method for analytical comparisons.

Results: For the candidate method, results indicated positive PCR detection within acceptable ranges for pathogen targets for all matrices screened. All candidate samples confirmed 100% with both alternative and traditional methods. Reference method sets also demonstrated 100% alignment with traditional culture plate confirmations and biochemical identification. All uninoculated controls tested negative for candidate target assays, alternative and reference confirmations. At $p<0.05$, POD analysis demonstrated no statistically significant differences between number of positives detected by candidate, alternative and reference methods. At a high level, five positives were detected irrespective of detection method with no statistically significant differences observed.

Significance: Results indicate that SLM, LIS and LMO assays are suited to testing challenging matrices such as food flavorings for the presence of *Salmonella* and *L. monocytogenes*.

P1-286 Identification of *Listeria* spp. and *Listeria monocytogenes* with Nanopore Sequencing-Based Whole Genome Sequencing

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Introduction: Currently within the food industry, for any *Listeria spp.* and *Listeria monocytogenes* positive results generated by conventional agar detection methods or rapid detection methods, the identity of the micro-organisms must be confirmed by a series of biochemical confirmation tests taking more than two – four days.

Purpose: To develop a more rapid and accurate confirmation method, providing genetic diversity information for true genesis possible further investigation as well, we investigated a rapid molecular-based *Listeria spp.* and *Listeria monocytogenes* confirmation method: nanopore-based whole genome sequencing (WGS) method.

Methods: We sequenced the whole genomes of 18 *Listeria spp.* isolates and 80 *Listeria monocytogenes* with GridION for 24 to 48 hours, with 5-7 isolates multiplexed in each flow cell. For the 18 *Listeria spp.* isolates (representing 16 *Listeria* species) plus 3 of the *Listeria monocytogenes* isolates, taking results from Illumina data as benchmark, we assessed the accuracy and efficiency of using nanopore data to identify the species of the isolates tested with ANIb, *SigB* allelic typing and Kraken2 method. For 77 of the *Listeria monocytogenes* SNP analysis and cgMLST analysis were carried out for genome comparison.

Results: We found that, nanopore WGS data could accurately identify all 17 *Listeria* species selected at 30 × depth of genome coverage or beyond. The accuracy was identical to using Illumina data or hybrid data (Illumina + nanopore) at 200 × depth plus of genome coverage. For genome comparison, nanopore data achieved comparable results to Illumina data in terms of differentiating *Listeria monocytogenes* with different level of SNP distance based on the records from NCBI Pathogen Detection database, using both SNP method and cgMLST method, at 200 x depth of genome coverage.

Significance: We demonstrated that nanopore-based WGS method can be used as a rapid confirmation method for *Listeria spp.* and genome comparison method for *Listeria monocytogenes* for the food industry.

P1-287 Miniaturized Devices for Point-of-Care Testing of Foodborne Pathogens

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Introduction: Nowadays, molecular methods for point-of-care testing have attracted the interest of the scientific community as a way to reduce the time to result and to better trace the food chain to avoid food fraud and to ensure food safety.

Purpose: We report the implementation of simple handheld device for DNA purification suitable for decentralized setups.

Methods: Fresh *E. coli* cultures were prepared in NB and used to spike ready-to-eat salmon. The samples were enriched overnight and then the DNA was extracted by using reagents from "RTP Pathogen Kit" from INVITEK, producing the lysis of the bacteria by incubating the samples with a mixture of proteinase K and lysozyme, at 50 ºC for 10 min. The DNA was purified by passing it through a microfluidic device, made of polymethylmethacrylate (PMMA), which consist of a W-shaped channel where silica beads of a size of 500 µm have been immobilized. Here silica beads accomplish the function of retaining DNA that has been previously released, allowing washing and elution of DNA using ethanol and RNAse-free water, respectively. The purified DNA was analyzed by Loop-mediated isothermal amplification (LAMP) with real-time fluorescence acquisition and by end-point colorimetric LAMP. Both detection methods were performed at 65 ºC for 45-75 min.

Results: With the simple handheld device, it was possible to obtain LAMP-amplifiable DNA with comparable quality to that of the commercial kit. When implementing the real-time fluorescence approach, positive results were obtained in 20 to 40 min, depending on the initial inoculation level, while with the colorimetric LAMP a longer incubation time was needed, 45 to 75 min, for a clearer color discrimination.

Significance: In most POCT little attention is paid to the sample processing step. The protocol, and device, reported herein have the potential to provide a low cost, simple to operate approach, for reliable pathogen detection in decentralized setups with low infrastructure.

P1-288 Detection of *Salmonella* spp. by Loop-Mediated Isothermal Amplification in Environmental Samples from Broiler Flocks

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Introduction: *Salmonella enterica* control in broiler farms is essential to prevent the spread of this pathogen throughout the food chain.

Purpose: To develop a simple and rapid method for the detection of *Salmonella enterica* in environmental samples from broiler flocks based on the isothermal LAMP technique.

Methods: To simulate broiler farm sampling, two pairs of boot swabs, with 25g of broiler bedding, were mixed with 225mL of BPW and incubated at 37h for 18h. One mL was used for DNA extraction. Four different protocols were compared: Thermal Lysis (TL) at 99ºC, Chelex, TL with magnetic bead purification, and TL with glass milk purification. The Samples were analyzed in parallel with ISO 6579:2017. DNA was analyzed with by fluorescent and colorimetric LAMP targeting the *ttr* gene. A total of 30 samples were used to calculate the Limit of Detection (LoD), and the method was validated with real samples and with samples for an interlaboratory test.

Results: Dynamic range assays showed that LAMP method detected up to 0.136 pg of pure culture DNA and 3.3 log UFC/g of *Salmonella* in feces samples. A total of 31 serotypes different serotypes and 3 *Salmonella* subspecies were included in inclusivity assays. The lowest LoD_{95} was obtained with TL with 7.99 CFU/sample. There was total concordance between ISO 6579:2017 and both Fluorescent and Colorimetric LAMP method in the interlaboratory test.

Significance: The method developed can be implemented at the point of interest, getting the results in only 24h.

P1-289 Design of a Selective Broth for the Co-Enrichment of *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes*, Suitable for Molecular Biology Applications

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Introduction: Multiplex methods are a straightforward way to increase throughput and reduce costs and hands-on work however, finding suitable culture conditions for several pathogens can be challenging, particularly if the food samples are heavily contaminated with interfering bacteria.

Purpose: Develop a selective broth for the simultaneous recovery of *Salmonella* spp., *E. coli* O157 and *L. monocytogenes*, suitable for molecular detection.

Methods: A non-selective media, previously reported suitable for the recovery of the target bacteria, mTA10 was selected. In other improve the recovery of stressed bacteria, and in particular *L. monocytogenes*, sodium pyruvate and cellobiose were added along with four selective agents (nalidixic acid, potassium tellurite, lithium chloride and sodium cholate). Growth kinetics were followed in real time at OD600 for 24h and the final concentration reached in mixed cultures and spiked ready-to-eat (RTE) salad samples were determined. Finally, RTE salad and salmon samples spiked at different concentrations with the tested pathogens were analyzed by multiplex qPCR and MinION amplicon sequencing.

Results: The addition of the growth promotors, along with the selective agents, allowed to obtain a selective broth capable of recovering all target bacteria without significant effect on the final concentration (7 log CFU/mL in mixed culture and more than 5 log CFU/ g in spiked salad samples). It was possible to detect below 10 CFU/ 25 or 10 g of spiked RTE salad or salmon sample with two different molecular methods, a tetraplex qPCR and by MinION DNA sequencing.

Significance: The novel broth presented herein can set the basis for the development of novel methods intended for the analysis of food samples with a high concentration of background bacteria. Additionally, it may be implemented in a semi-targeted DNA sequencing format for the detection of these, and other pathogens in one single sequencing analysis.

P1-290 Rapid Isolation and Detection of *Salmonella* on an Automated System MagiCyte MB

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Introduction: Colonies play a crucial role in test for *Salmonella* in food safety. However, conventional culture methods to isolate *Salmonella* from food is time consuming and resource intensive.

Purpose: This work aims to develop a high-throughput automated method for rapid isolation and detection of *Salmonella* from food.

Methods: We developed a MagiCyte MB device where users can load up to 96 samples for rapid isolation and detection of *Salmonella.* In this automated method, *Salmonella* cells are separated from the samples by immunomagnetic separation (IMS) and then plated in the wells in a standard 96-well microplate filled with selective agar. Time-lapse images of each well are automatically analyzed to detect microcolonies based on the changes in size and color, and to track discrete colonies. To validate the method, test portions of onion powder (25g each) in triplicates were inoculated with 1-2 *Salmonella* cells. After 16 hours of pre-enrichment, aliquots of each sample were mixed with anti-*Salmonella* antibody conjugated beads in a 96-well microplate and then loaded into the device for analysis. The four representative *Salmonella* strains recommended by FDA BAM were evaluated. *E. coli* inoculated and un-inoculated samples were used as negative controls.

Results: The time-lapse images clearly show the growth of the microcolonies and detects the microcolonies when they are as small as 50 µm. The isolation and detection of *Salmonella* from onion powder takes less than 28 hours (including 16 hours of pre-enrichment) for all samples inoculated with Salmonella. No colonies from the negative controls were detected.

Significance: The developed method obtains isolated Salmonella colonies for downstream analysis days sooner than conventional methods and provides labs an easy testing workflow with higher efficiency. By replacing Petri dishes with a single well in a microplate, this miniaturized microbial culture reduces media usage and bio-waste generation by a factor of more than 200. The technology can readily be adapted to test for other common foodborne pathogens.

P1-291 Rapid Detection and Quantification of Bacterial Cells Recovered on Food-Contact Surfaces Using a Smartphone Microscope

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❖ ***Developing Scientist Entrant***

Introduction: Surveilling bacterial cells on food-contact surfaces is essential to ensure food safety and mitigate the risk of the potential for foodborne-illness outbreaks. However, current methods face challenges. The plate count method is time-consuming and labor-intensive. The rapid adenosine triphosphate (ATP) method is associated with a lack of standardization and insufficient specificity to differentiate between bacterial ATP and food ATP.

Purpose: this study aims to develop a rapid, simple, cost-effective method to detect and quantify bacterial cells on food-contact surfaces.

Methods: The Salmonella enterica (SE1045) cells distributed on 10×10 cm^2 stainless-steel surfaces were collected by swabbing with the ethanol-moistened foam swab. The collected SE1045 cells were released into 300 µL absolute ethanol, and 5 µL of the suspension was dropped onto a prepared 3-mercaptophenylboronic acid (3-MPBA) coated gold chip which was fast evaporated within 20 seconds. The 3-MPBA binds to the glycans on the bacterial outer surface to achieve non-specific bacterial species detection and enables the visualization of individual bacterial cells under a smartphone light microscope which was purchased from online vendor for $ 30. Cell numbers were quantified by our developed smartphone application (BactiScan) with embedded ImageJ function.

Results: This method demonstrated good quantification capability for SE1045 cells on stainless-steel surfaces in the range of 10^3-10^7 CFU (r^2 = 0.9586) with the limit of detection as low as 2000 CFU/100 cm^2. The entire procedure (from swabbing to getting results) was accomplished within 5 minutes. The consumables (excluding the smartphone microscope) costs only $2 per sample. Moreover, our developed APP is currently available in the Apple Store for free download and use.

Significance: Our developed method presented a novel solution for rapidly assessing surface bacterial contamination. While it is still in an early exploration stage, our method exhibits great application and market potential for bacterial surveillance on food contact surfaces.

P1-292 An Integrated Approach of BAX® System PCR, ISO 22964 and MALDI-TOF for Rapid Detection of *Cronobacter sakazakii* in Food and Probiotic Products

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Introduction: Infant formula, baby cereal and probiotic products need to be tested for *Cronobacter sakazakii* to prevent foodborne infections, particularly in newborns. The objective of this research was to evaluate an integrated approach for rapid detection and confirmation of this pathogen.

Methods: Samples were enriched in BPW, and then in *Cronobacter* Selective Broth (CSB). The enrichments were tested using BAX® System PCR for *Cronobacter* (*E. sakazakii*), followed by culture isolation on chromogenic *Cronobacter* isolation agar (CCI) per ISO 22964 and colony confirmation using MALDI-TOF (VITEK® MS, BioMerieux). This integrated testing approach was validated using infant formula, cereal, and probiotic products inoculated with *C. sakazakii* at different levels.

Results: For infant formula and cereal samples (10g) inoculated at 12 CFU/sample (n=13), *Cronobacter* were detected, isolated and confirmed in all samples while uninoculated samples (n=6) were negative. For samples inoculated at 1.2 CFU/sample, fractional recoveries were achieved in infant formula

and cereal samples (n=12/14), indicating that the method was able to detect 1 CFU in these matrices. Significant matrix effect was observed for probiotic samples (n=16) in both the PCR and culture method due to potential antimicrobial activity of the products which was resolved by reducing the sample to BPW ratio to below 1:100 prior to initial enrichment. This allowed successful detection and isolation of the target in probiotic products by both BAX® PCR and ISO 22964 methods. Overall, the integrated method with PCR screening and culture confirmation resulted in 100% sensitivity, 95.2% specificity, 4.8% false positive and 0% false negative in a paired validation against the culture-based ISO 22964.

Significance: The integrated method of BAX® System PCR, ISO 22964 and MALDI-TOF provides an effective approach from detection to isolation and confirmation for rapid detection of *C. sakazakii* in infant formula, cereal and probiotic products to support food safety practices.

P1-293 Development of Multi-Residue Analytical Method for 17 Pesticides in Livestock Products Using LC-MS/MS

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Introduction: Livestock are exposed to pesticides on consumption of animal feeds made by grain and unintentional contamination from environment. Especially, it is necessary to anlayze the 2,4-D and 2,4,5-T for used as herbicides. Therefore, it is essential to develop a reliable and accurate multi-residue analytical method that can be applied to monitoring of residual pesticides in livestock products for food safety.

Purpose: This study was aimed for development and validation a multi-residue analytical method for 17 pesticides in livestock products (beef, pork, chicken, milk, egg and fat) using quick, easy, cheap, effective, rugged and safe (QuEChERS) method by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: Residual pesticides were extracted with acetonitrile followed by addition of $MgSO_4$, sodium chloride, Na_3 citrate and Na_2 citrate. Then, the extracts were cleaned up using $MgSO_4$, and C_{18}. To verify the applicability of the developed method, selectivity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) was evaluated.

Results: The coefficient of determination (R^2) was above 0.98 within the concentration range from 2.5 to 100 ug/L. The average recoveries were most in the range of 66~118% and standard deviation values were less than 16.5% at spiked levels of 0.01, 0.02 and 0.1 mg/kg which is satisfied the codes guideline (CODEX CAC/GL 40).

Significance: These results suggest that the validated method can be applied to the monitoring of livestock products and strengthen the food safety management.

P1-294 High Resolution *Salmonella* Detection, Serotyping, Subtyping, Surveillance, Differentiation of Multiple Serotypes and Quantification in a Single Tube and Single PCR by ChapterDx MLSTnext NGS Technology

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Introduction: There is a need for a comprehensive, cost-efficient and user-friendly assay to detect/screen, serotype, subtype, monitor surveillance, differentiate between multiple serotypes present in the same sample and quantify *Salmonella* serotypes in a single tube/PCR format all at once. The state-of-art methods require multiple procedures/methods, are expensive, complicated and time-consuming and many laboratories do not have the facilities/platforms to perform comprehensive analysis and rely on third party labs.

Purpose: To develop a high resolution, cost-efficient single tube and single PCR NGS technology with the capabilities of 1) detection/screening, 2) genotyping/serotyping, 3) subtyping, 4) detection of multiple genotypes/serotypes in the same sample, 5) quantification and 6) surveillance, and 7) integrated data analysis software.

Methods: The MLSTnext NGS technology amplifies and sequences up to 100-200 polymorphic loci in a single-tube and single-step PCR reaction, evenly spanning the *Salmonella* genome. In the workflow, amplification and barcoding/indexing of each sample occurs simultaneously in the PCR reaction. After Amplification, amplicons are pooled, and sequencing is performed by NGS. In this study, 15 (ongoing investigation with larger sample size) previously culture-confirmed samples of *Salmonella* (including ATCC isolates) were evaluated by MLSTnext technology.

Results: The assay generated high-resolution results that were able to detect and serotype each isolate to subtype level. The assay differentiated between two *S. typhimurium* strains by sequence differences in 4 loci, indicating the same serotype but different strains. Moreover, the assay could detect co-presence of multiple *Salmonella* isolates for 3, 6 and 9 different serotypes in the same sample. At the same time, the MLSTnext assay generated quantification results that were in agreement with real-time PCR. Moreover, the loci profile for each sample allowed to effectively track-and-trace the source of contamination for surveillance.

Significance: MLSTnext assay is the simplest NGS workflow, generating comprehensive results cost-efficiently and the entire workflow takes less than 12-24 hours (depending on the sequencing kit used).

P1-295 Rapid and Data-Efficient Classification of *Salmonella S*erovars Using Augmentation and Deep Learning on Hyperspectral Microscope Images

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Introduction: Addressing the need for rapid and accurate detection of foodborne pathogens, hyperspectral microscope imaging (HMI) has demonstrated promising results in classifying bacterial species at the single-cell level using their unique spectral signatures.

Purpose: This study aimed to enhance the utilization of spatial features in high-dimensional HMI data to enable serovar-level classification by developing image augmentation and deep neural network algorithms.

Methods: Darkfield HMI data of five different *Salmonella* serovars, including Enteritidis, Typhimurium, Kentucky, Heidelberg, and Infantis, were collected from pure bacterial isolates at the US National Poultry Research Center of USDA-ARS in Athens, GA. A total of 243 raw HMI data were pre-processed to extract spatial data using the Spectral Python module, followed by a standard 70/30 train/test split. An artificial intelligence (AI) model was developed based on a lightweight deep learning architecture for image classification (EfficientNet variant), pre-trained on the large ImageNet dataset, and subsequently trained with our HMI data for data-efficient transfer learning. Additionally, a combination of various image augmentation algorithms relevant to the data acquisition process (e.g., illumination, target orientations, focusing, geometric transformations, sensor noise, and missing information) was designed for bacterial datasets to enhance data efficiency and model generalizability. The model performance was evaluated on a held-out test dataset using a confusion matrix, accuracy, precision, and recall at different bacterial incubation times (6–24 h).

Results: Overall, the AI model prediction results for *Salmonella* serovar classification based solely on spatial data from raw HMI datasets demonstrated high accuracy, ranging between 0.80–0.99 depending on the incubation time. The results also indicated that the overall classification accuracy was improved up to 1.46-fold by employing image augmentation during model training.

Significance: This study supports the robustness of AI-assisted HMI as a data-efficient method for rapid and accurate foodborne pathogen detection at the serovar level.

P1-296 Sample-to-Answer Portable Device for Rapid Bacterial Detection in Food Products

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Introduction: Rapid detection of target bacteria with high sensitivity is vital for food safety. One of the key approaches to reducing detection time is to develop portable low-cost devices. These portable analytical devices combined with robust molecular approaches could provide sensitive and specific detection of target pathogens without significant labor and need for sophisticated laboratories.

Purpose: We aimed to develop a sample-to-answer device for rapid, simple, sensitive, and portable detection of bacteria within a single work shift (<8 h) in food industries using reporter phages.

Methods: We designed a phages-based biosensor that integrates all laboratory operations into a single, miniaturized device. The biosensor was fabricated using the standard laser-cutting technique. Bacterial samples were concentrated in the biosensor through in-device filtration. Phages T7-ALP were added to infect the target host bacteria *E. coli*, followed by the colorimetric enzyme reaction. The presence of *E. coli* was determined when there was a color change. The sensitivity of portable devices was evaluated by testing 10-fold serial dilutions of target *E. coli*. The detection performance was also validated for food products and compared to traditional culture-based methods. The color intensity was determined using the image analysis software ImageJ. One-way ANOVA was used to analyze the statistical differences among different groups.

Results: The biosensor contained multiple function components including a sample loading inlet, sample container, filtration hole, and absorbent pad holder. One-milliliter bacterial samples were concentrated in the device within 3 min. The detection limit was 50 CFU/mL of *E. coli* in pure culture medium with a total analysis time of 6 h. This biosensor could detect 500 CFU/mL of *E. coli* in apple juice or tap water within 6 h.

Significance: The phage-based portable biosensor benefits food industries and public health by early detecting contaminated food products, thus reducing food recalls and foodborne illness outbreaks.

P1-297 *Listeria monocytogenes* Rapid Typing Based on Fourier-Transform Infrared Spectroscopy (IR)

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Introduction: *Listeria monocytogenes* is a foodborne pathogen with its outbreak potential related to its serotype. Existing typing methods are both costly and time-consuming, prompting the quest for rapid and reliable alternatives. Fourier transform infrared spectroscopy emerges as a promising technique, involving bacterial exposure to infrared light and the creation of unique absorption spectra for each.

Purpose*:* Determinate the ability of the Fourier-transform infrared spectroscopy as a method to identify serogroups of *Listeria monocytogenes* strains obtained from different sources.

Methods*:* A total of 52 *L. monocytogenes* strains were examined in this study. Among them, 18 clinical strains were isolated between 2017 and 2023, 34 food strains including 12 isolates obtained from 2009 to 2010 (1 from ground meat, 2 from ice cream, 3 from ham, 1 from pork pate, 1 from cheese, and 4 from sausages), and 22 isolates obtained between 2021 and 2022 from farmed salmon. On these isolates, we compared the spectra generated by the IR Biotyper® equipment with classical serotyping.

Results*:* Using the Seeliger and Höhne method, 43 strains were classified into serogroup 1/2, while 9 belonged to serogroup 4. Each strain underwent triplicate processing and three runs, resulting in 464 spectra analyzed in this study. A confusion matrix assessed the equipment's identification accuracy based on the provider's training. The equipment correctly classified 100% of the strains, with 99% accuracy.

Significance: Infrared spectroscopy opens new tools for the serotyping of *L. monocytogenes*, allowing rapid and economical knowledge of the *L. monocytogenes* serogroups to determine their distribution in foods and the study of outbreaks. This method has then epidemiological value in the clinics and the food industry.

P1-298 Portable MSI Device for the Monitoring of the Fish Quality throughout Food Supply Chain

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Introduction: Monitoring food quality throughout the supply chain in a rapid and cost-effective way allows the on-time decision making reducing food waste and maintaining food sustainability.

Purpose: In that framework a portable multispectral imaging sensor was used, the acquired data in combination with neural networks was evaluated for the prediction of fish quality in different packaging conditions and fish parts.

Methods: Images of fish fillets were acquired from aquaculture, retail stores also small storage was conducted simulating consumer behavior (i.e., 720 images of flesh/skin). In parallel to image acquisition also microbial quality was estimated. The products were stored at different temperatures and packaging conditions. The models were developed and validated using the data from aquaculture and were externally validated with the samples purchased from the retail stores, aiming to estimate the TAC.

Results: The set up allowed the evaluation of models for the different parts of the fish and conditions. The performance for the validation set was similar for flesh (RMSE: 0.402-0.547) and skin side (RMSE: 0.500-0.533) of the fish fillets. Also similar were the performance for the different packaging conditions, but for the case of external validation the vacuum-packaged samples showed better performance in terms of RMSE compared to air-packaged. The need of predictive models irrespective of packaging condition is very important for cases where the products' history is unknown although the prediction capability was not as high as in the models per packaging condition. The models tested with unknown samples (i.e., retail stores) showed poorer performance (RMSE: 1.061-1.414) compared to the models validated with data partitioning (RMSE: 0.402-0.547).

Significance: Multispectral imaging sensor showed promising results for the rapid assessment of the microbiological quality of fish fillets for the different cases evaluated.

P1-299 Quality Parameters of Different Fish Species: A Case Study for Rapid Estimation of Fat Content

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Introduction: Quality characteristics vary highly between fish species. Therefore, rapid and accurate methods for the estimation of nutritional parameters are of great importance for food industries.

Purpose: The objective of this study is to determine the microbiological quality and nutritional parameters (fat and protein content) of different fish species and predict the total fat content using spectroscopic analyses, coupled with multivariate data analysis.

Methods: Samples from 7 fish species (n=120), cod, tuna, trout, seabass, sea bream, mackerel and salmon, were obtained from various fish markets. Approximately 5 batches were acquired with 4 fish samples per batch. A part of each fillet was homogenized through blending with a food processor, and stored at -20°C. The rest was used for microbiological analysis on the day of the collection. Total fat and protein content of each species was determined by Soxhlet and Kjeldahl method, respectively. The analytical procedures applied on the ground samples for the rapid prediction of total fat content included FTIR and NIR spectroscopy measurements and MSI acquisition. Stratified sampling was applied so as 75% of each dataset to be used for training and 25% for testing the models. Partial least squares regression was applied on the collected data for the prediction of total fat content after the spectral data were preprocessed.

Results: Microbiological results for all fish species showed a range of 2.00-5.53 log CFU/g for total viable counts. Prediction performance in terms of R^2 and RMSE of the test set for the FTIR measurements were 0.797, 1.937 and for NIR were 0.852, 1.638, respectively. Similar was the performance for the two examined MSI sensors (benchtop, R^2=0.808, RMSE=1.635), (portable, R^2=0.735 RMSE=1.919).

Significance: The collected information from this study showed potential and will be further investigated in terms of important features and prediction of nutritional quality.

P1-300 Fluorescence Fingerprints of Vegetable Juices: Monitoring Food Safety and Quality by Determining Treatment Efficacy and Remaining Shelf-Life

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❖ ***Developing Scientist Entrant***

Introduction: Fluorescence fingerprints, or excitation-emission matrices (EEMs), provides a simple, rapid, and comprehensive tool to scout progressive quality and safety risks in perishable products, such as highly consumed vegetable products.

Purpose*:* This study assessed the efficacy of EEMs to report on the safety and quality of spinach juices (untreated vs. pasteurized vs. antimicrobial agent) and subsequently, remaining shelf life.

Methods: Spinach was blended with deionized water (1:1 w/w) to prepare juice-like products. Each sample was divided into three aliquots (1-left untreated, 2-pasteurized (72°C, 15s), 3-with addition of gallic acid (8 mg/mL) and stored at 4 and 15°C for 7 days. Samples were tested daily, at least in duplicates. EEMs (λ_{ex}=250-530nm, λ_{em}=270-750nm, slits=2 and 3 nm) were collected using a spectrophotometer. Distinctive features (e.g., discriminative bands) of the EEMs were verified by LC-MS/MS. Microbial growth during storage was monitored by plating on Tryptic Soy Agar after serial dilution and incubating at 37ºC for 17 h.

Results: Three main EEMs regions exhibited most changes during storage for treated (n=28) and untreated (n=14) samples, albeit the extent of changes differ among treatments. LC-MS/MS corresponded these features with aromatic amino acids (AAC), polyphenols (PP), and chlorophyll a (ChA). A spoilage index (SI) based on ratio of the relative intensity of the ChA and PP bands ($(I_{680\ (Ex@420nm)}/I_{425(Ex@325nm)})$) and an ANN approach ($\lambda_{Em\ 325\text{-}700nm}$ @ $\lambda_{Ex\ 325nm}$ & $\lambda_{Em\ 420\text{-}700nm}$ @ $\lambda_{Ex\ 420nm}$) were compared for their ability to classify samples into fresh and spoiled (55 vs. 90% correct) according to the chemical (change in composition) and microbial growth results (e.g. from 7.5 to 8.9 log CFU/mL for untreated juice stored at 15°C). ANN allowed identifying early spoilage in both treated and untreated samples.

Significance: EEMs allowed for identifying unique and distinctive features to characterize treatment efficacy and spoilage in spinach products. This may improve shelf-life reporting, thereby ensuring that consumers receive safe and high-quality foods.

P1-301 Evaluation of Rapid ATP Bioluminescence Method for Microbial Detection in Highly Formulated Protein Drinks

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Introduction: Rapid ATP bioluminescence technologies, as an alternative to standard plating method, are aimed to verify commercial sterility of UHT aseptic products immediately after pre-incubation period, thus significantly shortening time needed for determination of microbial contamination and resulting in cost saving due to faster release of the products.

Purpose: This study was to evaluate End Product Indicator-Charm (EPIC) method for detecting microbial contamination in highly formulated protein beverages.

Methods: 680 of samples of beverages with different protein sources, including milk, whey, pea, pumpkin, oat, flaxseed, sesame proteins and combinations thereof, were inoculated with low levels of 8 microorganisms: *Clostridium sporogenes* ATCC 11437, *Listeria innocua* ATCC 33090, *Staphylococcus aureus* ATCC 51153, *Bacillus subtilis* ATCC 55614, *Salmonella enterica* ATCC 14028, *Bacillus coagulans* ATCC 7050, *Pseudomonas aeruginosa* ATCC 27853 and *Geobacillus stearothermophilus* ATCC 10149. Inoculated samples (n=3 per each inoculum level per microorganism) were incubated along with the negative controls at 30°C (55°C for *G. stearothermophilus*). Aliquots were aseptically taken out from the samples after 24, 48 and 72 hours of pre-incubation and tested on EPIC (n=3), as well as pour plated onto appropriate agar for detection of microbial growth.

Results: Reagents and luminometer used for this study were able to detect microbial contamination with each of 8 microorganisms in all 14 tested types of products after 24-48 hours of pre-incubation at less than 10 CFU/container initial inoculum level. Bioluminescence results correlated *p*=1.0 with results obtained by standard plating method using media and conditions appropriate for each tested strain.

Significance: Evaluated method can reliably detect low level microbial contamination in aseptic highly formulated protein drinks equivalently to reference method, but after only 24-48 hours of incubation. This allows earlier detection and faster release time for the products compared to traditional microbiological methods.

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P1-302 Developing a Surface Plasmon Resonance Biosensor for the Quantification of *Salmonella* Typhimurium in Ground Chicken

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❖ ***Developing Scientist Entrant***

Introduction: Regulatory bodies in many countries have implemented measures to monitor and reduce *Salmonella* contamination in poultry products. Rapid quantitative analysis enables producers to identify potential issues in their production processes, allowing for corrective measures to reduce *Salmonella* levels and improve overall product safety.

Purpose: This study was to develop a Surface Plasmon Resonance (SPR) biosensor for the quantification of *Salmonella* Typhimurium in ground chicken and to assess the performance of SPR in comparison with real-time PCR (RT-PCR).

Methods: SPR biosensor was functionalized with a well characterized monoclonal antibody specific to the flagellin. A SPR workflow was optimized for quantitative determination. Ten sets of 6 ground chicken samples (32.5 g) were inoculated with a cocktail of 4 strains of *S*. Typhimurium at target levels between 0 and 4 log CFU/g. Samples were analyzed by SPR and RT-PCR following buffered peptone water (BPW) and BAX MP Supplement (MPS) enrichment for 6, 8, 10, and 12 h at 42 °C.

Results: SPR responses (μRIU) demonstrated a log-linear correlation with the concentration of *S*. Typhimurium within the range of 4.6×10^5 and 2.1×10^7 CFU/mL. The use of MPS for the enrichment of *Salmonella* in chicken samples proved equally effective for both SPR and RT-PCR. A linear regression was performed for each enrichment time. The optimal linear fitting between 0 and 4 log CFU/g was observed at 10 h of enrichment ($R^2 \geq 0.92$) for SPR, as opposed to 6 h of enrichment ($R^2 \geq 0.90$) for RT-PCR. Limit of quantification (LOQ) for SPR was determined to be 0.77 log CFU/g, and there was no significant difference ($p < 0.05$) when compared with RT-PCR.

Significance: The results indicate that the accuracy of SPR in estimating *S*. Typhimurium in ground chicken is comparable to that of RT-PCR. The cost-effectiveness of SPR makes it an appealing alternative for enumerating *Salmonella* in poultry products.

P1-303 Development of a Sequencing-Based Strategy as a Confirmatory Method for Detection of *Cyclospora cayetanensis*

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Introduction: *Cyclospora cayetanensis* is a coccidian parasite that causes the gastrointestinal illness cyclosporiasis. This parasite is an important cause of foodborne outbreaks, associated with the consumption of fresh produce and contaminated irrigation water.

Purpose: The intention of this study is to develop a confirmatory method for *C. cayetanensis* detection based on sequencing of conventional-PCR amplicons. Two markers targeting the mitochondrial genome, different from the recently described Mit1C, were evaluated regarding the ability to sequence positive samples that have varying DNA amounts.

Methods: DNA from purified clinical samples was used. Twenty samples were tested using qPCR with Mit1C for detection of *C. cayetanensis* and divided into four groups based on Ct-values. Two separate conventional PCR reactions were also performed, one for each marker, with those samples, using touchdown protocol; amplicons were visualized in QIAxcel Advanced System. Purification utilized the QIAquick PCR Purification Kit and concentrations were obtained with Qubit. All samples underwent Sanger-sequencing, were assembled using SeqMan Ultra 17, and subjected to phylogenetic analysis against reference sequences.

Results: All 20 samples yielded 100% positive results and Ct values between 28-38 for Mit1C. Sample DNA concentrations ranged from 0.176 to 23 ng/μL. Sequencing confirmation of the amplicons with the two additional markers was obtained from all samples from groups 1-3 corresponding to Ct values of 28-36. Group 4 samples provided partial confirmation due to fractional levels according to the FDA Microbiological Methods Validation Subcommittee. Phylogenetic analysis confirmed sequences were specific to *C. cayetanensis*.

Significance: Sequence confirmation of non-culturable pathogens is challenging especially when low amounts of DNA are available. Development of a sequencing-based strategy for confirmation of *C. cayetanensis* is of great importance to support regulatory results based on standard detection method (BAM Chapters 19b and 19c). These results support public health and the FDA mission to support findings requiring regulatory actions.

P1-304 Development of and Optimization of Crystal Diagnostics Xpress™ S Kit AOAC *Performance Tested Method*™(PTM 051602) for Detection of *Salmonella* spp.

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Introduction: *Salmonella enterica* (*S. enterica*), found in a variety of foods including meat, fruit, vegetables, and processed foods, is recognized as one of the most common foodborne pathogens. The low infection limit (1 CFU) of *Salmonella* also posts high risk on consumers. There is a continuous demand for the development of highly sensitive and accurate routine test for *Salmonella.*

Purpose: The purpose of this study was to provide a fully automated *Salmonella* assay capable of processing large numbers of samples (high throughput) while ensuring that the Crystal Diagnostics (CDx) AutoXpress AXSALM test kit maintains the sensitivity and specificity of the previously accredited CDx Xpress S test kit.

Methods: A fully automated liquid crystal-based immunoassay was developed and optimized to detect *Salmonella* in foods. This study assay used different sizes (1uM and 3uM) of microspheres (capture and amplification) coated with three different commercially available antibodies. Semi-quantitative results were produced using the optical character of liquid crystal transformation.

Results: The results showed that the CDx AutoXpress AXSALM kit detected 101 unique *Salmonella* enterica subspecies or serovars (105 total strains tested) and 31 of the common foodborne pathogens were confirmed to not cause interference with the assay. Triple washed ready to eat baby spinach, beef trim and raw ground beef were used for matrix study to confirm that this assay can detect as low as 1 CFU per sample (200g, 200g, and 325g for spinach, beef trim, and raw ground beef respectively) after 16 hours of enrichment.

Significance: This study demonstrated that the CDx AutoXpress AXSALM kit is a rapid and accurate test for *Salmonella* spp. in foods. Other types of matrices will be investigated to discover further potential of this assay.

P1-305 Flow Cytometry for Paraprobiotics: Quantification of Inactivated Cells

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Introduction: Paraprobiotics are inactivated microbial cells or cell fractions which confer a health benefit to the consumer. The inactivated or dead cells in paraprobiotics cannot be quantified by traditional microbiological approaches. To assure quality in consumer products, another method of quantification is required. Flow cytometry (FC) is a promising method as it allows for direct quantification of injured and dead cells (non-Active Fluorescent Units or n-AFU/g).

Purpose*:* The purpose of this study is to develop an FC method to quantify inactivated cell levels in both paraprobiotic raw materials and complex fin-

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ished products with a high level of robustness and repeatability.

Methods: Three purchased raw material lots of heat-killed *Bifidobacterium* cells and a single lot of manufactured paraprobiotic finished product containing botanicals and heat-killed *Bifidobacterium* cells were analyzed in triplicate using an Attune NxT Flow Cytometer. Powdered samples were rehydrated in 0.9% saline and then serially diluted in 0.9% saline to achieve appropriate levels for analysis. Samples were stained using Sytox to differentiate dead cells from noise and other product components.

Results: The raw material lot used to create the finished product had a % relative standard deviation (%RSD) of 1.26 and ranged from $2.93x10^{11}$ to $3.00x10^{11}$ n-AFU/g (11.46687-11.47712 Log n-AFU/g). The method allowed for analysis of the finished product that reduced noise and plant cells from analysis while providing an average %RSD of 3.9 and a concentration range of $1.04x10^{10}$ to $1.37x10^{10}$ n-AFU/serving (10.01703-10.13672 Log n-AFU/serving) with an expected concentration of $1x10^{10}$ cells/serving (10 log cells/serving).

Significance: This work identified an analysis protocol that can be used to ensure quality of both raw materials and complex finished products. The flow cytometry method developed here can provide product confidence to both manufacturers and customers.

P1-306 Rapid Commercial Sterility Testing by bioMérieux D-COUNT® in Plant-Based Beverages and Chicken Broth

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Introduction: The bioMérieux D-COUNT® system (alternative method) combines fully automated fluorescent viable cell labelling and digital flow cytometry into a powerful system that can detect and count individual cells directly without the need for cell growth during the assay. It has been applied for the rapid testing of commercial sterility in aseptic food and beverage products.

Purpose: This study's objective is to determine the analytical performance of the alternative method for the commercial sterility test in plant-based beverages and chicken broth produced at a single manufacturing site.

Methods: Four plant-based beverages and one chicken broth were analyzed for commercial sterility using the alternative method. Fifteen samples were analyzed without inoculation and 130 samples were analyzed after inoculation with 3 different microorganisms *(Pseudomonas aeruginosa, Bacillus subtilis, or Enterococcus faecalis*). The alternative method and spread plate were performed after 3-, 4-, or 5-days pre-incubation.

Results: All uninoculated samples showed negative results which indicates the compatibility of these products with this commercial sterility test. The alternative method demonstrated 100% agreement with spread-plate confirmation across all pre-incubation time points. This indicates that the tested products have no interference with the alternative method detection and confirms the minimum pre-incubation needed for detection. The analytical performance of the alternative method is equivalent to the spread plate while giving the result 4 days sooner than the current method used by this producer.

Significance: The alternative method provides rapid microbial detection that enabled this aseptic producer to release final products 4 days earlier than their current method. Additionally, this rapid solution will help reduce costs by reducing hold time, decreasing inventory costs, and minimizing the risks and costs associated with potential in-process contamination.

P2-01 Efficacy of Orange Terpene against *Escherichia coli* Biofilm on Beef and Food Contact Surfaces

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Introduction: *Escherichia coli* (O157:H7) is a common pathogen found on food contact surfaces (FCS) and food surfaces (FS), which can cause foodborne illnesses. Biofilm formation by *E. coli* on FCS is a major concern for the food industry.

Purpose: The study aims to assess the effectiveness of orange terpene (OT) against *E. coli* biofilm on diverse food contact surfaces. Assessing surfaces like stainless steel, polyethylene terephthalate, low-density polyethylene, rubber, and beef, the research demonstrates significant reductions in 48-hour biofilm at various concentrations of orange terpene.

Methods: In controlled conditions, we cultured strains of *E. coli* and prepared surfaces for biofilm formation. Following this, we determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). We evaluated the impact of OT on both bacteria and surfaces, measuring parameters such as biofilm inhibition, hydrophobicity, auto- aggregation, and ATP levels. Additionally, we conducted analyses on color and texture and assessed biofilm structure using confocal laser scanning microscopy. Statistical analysis was then employed to evaluate the results.

Results: OT significantly reduced *E. coli* biofilm on diverse food contact surfaces, achieving1.4 to 2.09 log CFU/cm2 reductions. On beef surfaces, a 1.5 log bacterial reduction was noted at the minimum inhibitory concentration. Positive impacts on color and texture were observed, but varying concentrations had a negative odor effect. Electron microscopy supported the promising biofilm reduction, indicating OT as a potential natural solution for *E. coli* biofilm control in the food industry.

Significance: This study's significance lies in demonstrating orange terpene's efficacy in significantly reducing *E. coli* biofilm on diverse food contact surfaces. Positive effects on color and texture, despite odor impact, suggest orange terpene as a promising natural solution for controlling E. coli biofilm, offering potential advancements in food safety practices within the industry.

P2-02 Chlorine Dioxide's Antimicrobial Efficacy is Not Effected by Agriculture Water Quality when Treating Shiga-Toxin Producing *Escherichia coli* (STEC)

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Introduction: Agriculture water is treated with chlorinated disinfectants to prevent outbreaks. The efficacy of chlorine dioxide (ClO2), can be impacted by water quality and may influence industrial applications.

Purpose: The objective of this study is to determine if ClO_2 antimicrobial efficacy is dependent on the quality of the water being treated.

Methods: The minimum inhibitory concentration (MIC) of ClO_2 to achieve a 3-log reduction against STEC was determined in double deionized water (control), environmental samples from the Salinas Valley, and Environmental Protection Agency (EPA) designated water standards. EPA water was prepared following their protocol and adjusted to pH 6.5 and 8.4. Using the test waters, a stock ClO2 solution was diluted in a 24-well plate. A 7-strain STEC cocktail was inoculated into each well and sampled after 5 minutes. Each sample was plated on Sorbitol-MacConkey Agar for viable cell counts.

Results: We observed no significant difference in reduction between sterile double deionized control and sampled water, including EPA waters of 6.5 and 8.4 pH (N=3; p >0.05). Environmental samples with higher pH, turbidity, total dissolved solids, and conductivity showed a slightly lower reduction, although not significantly (p>0.05). Overall, we observed that while conductivity and turbidity may have influenced the MIC of ClO_2 against STEC, there was no significant difference in reduction across water samples.

Significance: ClO2 may be a useful alternative to conventional chlorine-based sanitizers because of its efficacy in different water qualities. Growers can use this knowledge to target pathogens in multiple water sources associated with agriculture.

P2-03 Prevalence and Genomic Antimicrobial Resistance of *Salmonella* and *Escherichia coli* from Retail Poultry in Southern California

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Introduction: The presence of antimicrobial-resistant bacteria in poultry meat is a public health concern, emphasizing the need for comprehension of its epidemiology.

Purpose: This study aimed to determine the prevalence and antimicrobial resistance (AMR) of *Escherichia coli* and *Salmonella* from retail poultry meat in California.

Methods: Retail poultry (141 chickens and 141 ground turkey) were collected from randomly selected grocery stores in southern California in 2020 and 2021 by the National Antimicrobial Resistance Monitoring System (NARMS) program. Samples were tested for *E. coli* and *Salmonella* using the NARMS Retail Meat Isolation Protocol. Serotypes of *Salmonella* and antimicrobial resistance genes (ARGs) in both bacteria were identified using whole genome sequencing.

Results: The prevalence of *E. coli* and *Salmonella* was 50.71% (143/282) and 15.25% (43/282), respectively. Twenty-seven (9.57%) samples were culture-positive for both *E. coli* and *Salmonella*. The prevalence of *E. coli* was higher ($p < 0.001$) in turkey (66.67%, 94/141) than in chicken (34.75%, 49/141). The prevalence of *Salmonella* was higher in chickens (17.02%, 24/141) than in turkey (13.48%, 19/141), but the difference was not significant ($p > 0.05$). Also, there was no significant year-wise variation in the prevalence of the bacteria. Whole genome sequencing revealed 13 distinct serotypes among the 43 *Salmonella* isolates, in which the top serotype was *S.* Kentucky (37.21%). Nineteen ARGs were identified in *Salmonella*, including bla_{TEM}, $bla_{CTX-M-65}$, and bla_{CMY-2}, responsible for beta-lactam resistance, along with a *gyrA* mutation (D87Y) associated with resistance to nalidixic acid and decreased susceptibility to ciprofloxacin. The *E. coli* isolates harbored 32 unique ARGs, including genes conferring resistance to beta-lactams, tetracyclines, aminoglycosides, and folate pathway antagonists.

Significance: The results illuminate the presence of foodborne bacteria and AMR in raw poultry and the necessity of proper handling and cooking. Results also highlight the significance of the ongoing retail food surveillance.

P2-04 Enhancing in vitro Inactivation of Escherichia coli ATCC 33625 by Sequential Application of Alkaline and Acidic Electrolyzed Water

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Introduction: Various strains of *Escherichia coli* are significant foodborne pathogens and electrolyzed water (EW) is a potent bacterial disinfectant which is safe and cost-effective; however, sequential application of EW varieties has seldom been studied.

Purpose: The research aimed to assess the effectiveness of alkaline electrolyzed water (ALEW), acidic electrolyzed water (AEW), and their sequential application in inactivating pure cultures of a nonpathogenic *E. coli* surrogate.

Methods: Bacterial inactivation treatments involved placing 1 ml stock culture (*Escherichia coli* ATCC 33625) into 9 ml of each generated solution (ALEW or AEW). After 1 min agitated incubation, 1 ml of each mixture was transferred into 9 mL of same-type treatment solution and incubated for another 1 min. A separate 'Mixed EW' treatment involved incubation of 1 ml stock culture with 9 mL ALEW for 1 min, followed by transferring 1 ml into 9 mL AEW and incubating 1 min. After 2 min, all samples were neutralized using 0.5% sodium thiosulphate, serially diluted, pour-plated, and incubated prior to counting colonies. Experiments were conducted in triplicate and data were statistically analyzed using ANOVA and Tukey's studentized range test ($p < 0.05$).

Results: AEW, ALEW, Mixed EW, and deionized water (DIW; control) had respective pH values of 2.54±0.01, 11.56±0.02, 3.51±0.46, and 6.54±0.76. Available chlorine content (ACC; mg/L) of AEW, ALEW, Mixed EW, and DIW was 64.58±12.41, 1.55±0.70, 52.43±11.77, and 0.00±0.00, respectively. Mixed EW significantly reduced *E. coli* counts by 7.17±0.2 log CFU/mL, while AEW, ALEW, and DIW had respective reductions of 2.00, <1, and 0 log CFU/ml.

Significance: The enhancement of in vitro inactivation of nonpathogenic E. coli by sequential application of ALEW and AEW paves the way for future work with pathogens in food systems.

P2-05 Transcriptional Analysis of *Escherichia coli* O157:H7 and a Non-Pathogenic *E. coli* in Response to the Chlorine Treatment Regulated by the U.S. Environmental Protection Agency

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Introduction: Sodium hypochlorite is a sanitizer, a powerful oxidizing agent, commonly used for treating irrigation water. However, its potency may be compromised by various environmental conditions, including water turbidity, pH, and temperature, likely contributing to recent *Escherichia coli* O157:H7 outbreaks in leafy greens.

Purpose: Since the bacterial response to attenuated chlorine effects remains unclear, the objective aims to understand transcriptomic mechanisms regarding the concentration of sodium hypochlorite, regulated by the U.S. Environmental Protection Agency (EPA), used for water treatment to improve the efficacy of chlorination.

Methods: *E. coli* O157:H7 strain (RM19259) and non-pathogenic *E. coli* (TVS353), both with 6 log CFU/ml, were individually treated with 4±0.2 ppm chlorine solution for 5 and 15 minutes, and the bacterial cultures without chlorination served as a control (also time 0). Both control and treated samples were added with chlorine neutralizer before being subjected to bacterial quantification and RNA extraction for sequencing and transcriptome analysis. These methods were performed in triplicate.

Results: The results showed that both *E. coli* O157:H7 and TVS353 had minimal reductions (0.3 to 0.5 log CFU/ml) after chlorine treatment regardless of exposure time. Transcriptome analysis revealed that treated TVS353 had a rapid quenching of oxidative stress from free chlorine to prevent irreversible damage in proteins and DNAs. Furthermore, the chlorine treatment activated physiological adaptations such as biofilm formation, osmotic adjustment, and potential deactivation of chlorinated compounds. Post-translational modifications by succinylation were evident, possibly resulting from neutralizing positively charged residues targeted by the treatment. In contrast, the treated *E. coli* O157:H7 exhibited minimal responses to the same chlorine treatment, highlighting strain-specific differences in adaptation and survival under chlorine exposure.

Significance: Current findings suggest that hurdle antimicrobial strategies are necessary to enhance the efficacy of the existing chlorination to prevent foodborne pathogens.

P2-06 Effect of Carvacrol Encapsulated into β-Cyclodextrins on the Survival of *Escherichia coli* O157:H7 during Common Storage and Cooking Practices of Beef Patties

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Introduction: Carvacrol, the main component of oregano essential oil, is well known for its strong antimicrobial effect against foodborne pathogens such as *Escherichia coli* O157:H7. Its hydrophobicity, strong flavor, and high volatility impede its commercial application in food preservation. Encapsulation of carvacrol into matrixes such as β-cyclodextrins (CDs) is considered an alternative antimicrobial intervention that may protect from oxidation and volatilization in addition to enhancing its bioactivity by providing a controlled and targeted release.

Purpose: This study aimed to evaluate the effect of carvacrol encapsulated into β-cyclodextrins (Carvacrol-βCDs) on the survival of *Escherichia coli* O157:H7 under consumer preparation practices of beef patties (i.e., cooking frozen vs cooking of refrigerated patties).

Methods: Inoculated (10^7 CFU/g) ground beef containing 1% Carvacrol-βCDs was shaped into beef patties (90 g). The patties were either cooked without freezing or after storage at -22°C for 7 days and cooked without thawing. Patties were cooked in pan-grill to internal temperatures of 62°C or 71°C. The above procedures were performed twice, and the samples were analyzed in triplicate.

Results: The addition of carvacrol-βCDs to beef patties did not affect the initial levels of TVC of patties, but significantly reduced the levels of *E. coli* O157:H7. Cooking of raw patties (without freezing) enhanced the survival of *E. coli* O157:H7 compared to cooking of frozen patties for 7 days. Cooking in pan-grill at 71°C was more effective against *E. coli* O157:H7 compared to cooking at 62°C. The addition of carvacrol-βCDs significantly reduced the levels of *E. coli* O157:H7 when cooked from frozen at 71°C (> 5 log CFU/g reduction) compared to cooking at 62°C (~ 3 log CFU/g reduction).

Significance: Results are indicative of the potential application of carvacrol encapsulated into β-cyclodextrins (Carvacrol-βCDs) as an effective antimicrobial treatment for reduction of *E. coli* O157:H7 in beef patties.

P2-07 Antibiotic Resistance of *Escherichia coli* Isolated from Green Leafy Vegetables in Accra, Ghana

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Introduction: *Escherichia coli*, an indicator of fecal contamination and potential presence of enteropathogens in foods has often been associated with leafy greens consumed raw or minimally processed.

Purpose: The study assessed the phenotypic profile and antibiotic resistance determinants of *E. coli* isolated from green leafy vegetables in Accra, Ghana.

Methods: Fecal coliforms (n=325) isolated from four Ghanaian indigenous and two exotic green leafy vegetables (n=91) in a previous study from farms and markets in Accra were screened for *E. coli* using biochemical tests. The antibiotic resistance profile of selected *E. coli* isolates (n=61, from 61 vegetables) to 11 antibiotics was determined using the Kirby-Bauer test. Minimum inhibitory concentrations (MICs) of the five most resisted antibiotics were determined using the twofold macro-dilution method. PCR was used to detect the presence of integrons and genes associated with ampicillin (*bla*-$_{CTX-M}$, *bla*-$_{SHV}$, *bla*-$_{TEM}$), sulfisoxazole (*sul1, sul2, sul3*), tetracycline (*tetA, tetB, tetC*), and streptomycin (*strA, strB*) resistance in the *E. coli* isolates. Plasmids were sequenced using the Oxford Nanopore technology.

Results: The *E. coli* isolates were resistant to two or more of the antibiotics used and 82% of isolates were multi-drug resistant. Ampicillin, sulfisoxazole, and tetracycline were antibiotics most resisted by 95%, 90% and 64% of the isolates respectively. MICs were 2-128 µg/mL for trimethoprim, cefoxitin, and streptomycin, 2-256 µg/mL for ampicillin and tetracycline, and 64-1028 µg/mL for sulfisoxazole. The *str*A, *str*B, *tet*A, *tet*B, *bla*-$_{CTX-M}$, *bla*-$_{TEM}$, and *bla*-$_{SHV}$ were present in the isolates, whilst *sul1, sul2, sul3, and tet*C were absent. Integrons were absent while plasmids were present in 76% of the isolates. The plasmids from six sequenced isolates harbored tetracycline, beta-lactam, streptomycin, sulfonamide, trimethoprim, and quinolone-related genes.

Significance: *E. coli* isolated from the vegetables carried genes associated with antibiotic resistance. Vegetable value chain actors must be encouraged to adopt measures that minimise microbial contamination of vegetables to improve food safety.

P2-08 Application of a Natural Antimicrobial Coating on Hass Avocados and Mangos for the Control of *Listeria monocytogenes* during Packaging

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Introduction: Natural antimicrobial coatings are used as a control measure in food products to reduce targeted microorganisms and ensure safety.

Purpose: To test a food-grade acetogenin-enriched avocado seed extract (ACE) mixed with propylene glycol (PG) as a coating to reduce *Listeria monocytogenes* on avocados and mangos, as a safety control measure.

Methods: Fresh avocados and mangos were coated with ACE before and after surface inoculating with *L. monocytogenes* at a target concentration of 4-5 log CFU/cm^2. The ACE concentration in the coating was 10,000 mg/L. Treatments included ACE coating and PG coating as a control, both applied before and after inoculation of the fruit. Inoculated, non-treated fruits were used as a positive control. For all treatments, core samples of the rind of these fruits were excised using a borer. Samples were homogenized in stomacher and spread-plated onto modified oxford agar for 48h at 37°C for *L. monocytogenes* enumeration. Counts were compared for bacterial reduction between treatments.

Results: Compared to counts on non-treated controls, the ACE reduced *L. monocytogenes* on avocado and mango coated before inoculating by 3.7 and 2.4 log CFU/cm^2, respectively. On these fruits coated after inoculating the reductions were by 3.4 and 1.2 log CFU/cm^2, respectively. PG also exhibited reduction on avocado and mango coated, before inoculating by 2.0 and 0.6 log CFU/cm^2, and after inoculating by 2.1 and 0.4 log CFU/cm^2, respectively. Considering that PG exhibited antimicrobial activity, the total reduction by ACE before inoculating on avocado and mango was 1.7 and 1.8 log CFU/cm^2, and after inoculating was 1.3 and 0.8 log CFU/cm^2, respectively. Results indicate that ACE extract can be potentially used to prevent or reduce *Listeria monocytogenes* in these fruits.

Significance: According to the results, the use of ACE extract as a coating has a future as an additional hurdle to the control of *Listeria monocytogenes* in fruit packing.

P2-09 Cannabidiol Modulates *Listeria monocytogenes* Proteome and Protects *Galleria mellonella* against Listeriosis

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Introduction: *Listeria monocytogenes* is a major pathogen causing foodborne illnesses in the US. Emerging antibiotic resistance in *L. monocytogenes* necessitates novel strategies for controlling listeriosis in humans.

Purpose: Our previous *in vitro* research revealed that cannabidiol (CBD), a non-psychoactive cannabinoid from *Cannabis sativa* attenuated *L. monocytogenes* virulence factors. This study investigated the effect of CBD on LM Scott A proteome and assessed its efficacy in protecting *Galleria mellonella* (insect model) against LM infection.

Methods: *L. monocytogenes* Scott A was incubated with or without CBD at subinhibitory concentration (SIC, 11.92 µM) or 6x SIC (71.52 µM) at 37°C for 24 h. Protein extractions from control and CBD-treated *L. monocytogenes* (n=3) were analyzed by UPLC-MS/MS to identify differentially abundant proteins.

Galleria mellonella larvae, injected with and without CBD at SIC and 6x SIC, were monitored for survival at 24h intervals for 7 days. Statistical analysis was performed using ANOVA (p<0.05, n=6).

Results: *L. monocytogenes* proteome analysis revealed that out of total 1736 proteins, CBD at SIC downregulated 31 and upregulated 20 proteins, whereas at 6x SIC, CBD downregulated 289 and upregulated 703 proteins (p<0.05). Downregulated proteins include those associated with *L. monocytogenes* virulence, including intracellular survival (plcA), flagellar synthesis (flaA, lmo0685), catalytic activity (glyQ, aspS), energy metabolism (pdhB, citG), fatty acid metabolism (accA, accD) and translation (rplA, rpsG). Upregulated proteins included those involved in peptidoglycan biosynthesis (murF, murG), energy metabolism (pflB), cell morphogenesis (minC, minD) and DNA repair (uvrB) (p<0.05). In *Galleria mellonella*, CBD protected larvae against *L. monocytogenes* infection, where 40% and 50% increase in survival were observed at SIC and 6x SIC, respectively compared to controls on the seventh-day post-infection (p<0.05).

Significance: Cannabidiol modulated LM proteome, indicating potential antilisterial effects. Cannabidiol also provided significant protection against *L. monocytogenes* in *Galleria mellonella*. Cannabidiol could potentially be used to control *L. monocytogenes* infection in humans. However, these findings warrant follow-up investigations in appropriate animal models.

P2-10 Multi-Species Biofilms Comprised of Environmental Microbiota Isolated from Fruit Packing Facilities Promoted Tolerance of *Listeria monocytogenes* to Benzalkonium Chloride

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Introduction: Formation of complex multi-species biofilms may promote the survival and persistence of pathogenic *L. monocytogenes* in food processing environments. However, it remains unknown whether biofilm formation by environmental microbiota increases the tolerance of *L. monocytogenes* to commonly used sanitizers, such as benzalkonium chloride.

Purpose: This study aimed to determine the ability of bacterial families previously shown to co-occur with *L. monocytogenes* in tree fruit packing facilities to form biofilms in single- and multi-family assemblages, and to assess the effect of the formed biofilms on the tolerance of *L. monocytogenes* to a sanitizer.

Methods: Biofilms were grown using individual families and multi-family assemblages on polystyrene pegs submerged in R2A broth for 3 days at 15°C. Biofilm formation was quantified using a crystal violet assay, spread plating, and amplicon sequencing. The concentration of *L. monocytogenes* in biofilms was determined using the most probable number method. Biofilms and planktonic cultures were exposed to 12.5 ppm of benzalkonium chloride, and the death kinetics of *L. monocytogenes* were quantified using a most probable number method.

Results: A total of 8, 8, 6, and 3 strains of *Pseudomonadaceae*, *Xanthomonadaceae*, *Microbacteriaceae*, and *Flavobacteriaceae*, respectively, isolated from the environmental microbiota of tree fruit packing facilities were used. Biofilms formed by *Pseudomonadaceae*, *Xanthomonadaceae*, and all multi-family assemblages had significantly higher concentration of aerobic mesophilic bacteria (p=1.12*10^{-6}), as well as *L. monocytogenes* (p=1.27*10^{-10}), compared to biofilms formed by *L. monocytogenes* alone. Furthermore, multi-family assemblage biofilms increased the tolerance of *L. monocytogenes* to benzalkonium chloride compared to *L. monocytogenes* mono-species biofilms and planktonic multi-family assemblages.

Significance: These findings suggest that *L. monocytogenes* control strategies should focus not only on assessing the efficacy of sanitizers against *L. monocytogenes*, but also against biofilm-forming microorganisms, such as *Pseudomonadaceae* or *Xanthomonadaceae,* that reside in the food processing-built environment.

P2-11 Extract from *Prunus spinosa L*. Kills *Listeria monocytogenes* upon Photosensitization with Red Light

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Introduction: *Prunus spinosa* L. (PSL) is a shrub that belongs to the rose family. It is commonly found in Europe, Africa, and Asia, and has long been used in diet and herbal medicine. The fruits of the PSL are known to have health-promoting and antimicrobial properties. However, their photosensitizing properties that could help fight foodborne pathogens are largely unclear.

Purpose: A combinatory approach using PSL and red light (RL) is investigated here to eradicate *Listeria monocytogenes*.

Methods: The extract from PSL at a subinhibitory concentration of 1 mg/mL was added to the cell suspension of *L. monocytogenes* ATCC15313 (10^8 CFU/mL), previously used in antimicrobial testing. The treated cells were subjected to 60-minute irradiation with an LED array at 630 and 660 nm, delivering a dose of 140 J/cm². After exposing the cells to RL, a standard microbiological analysis was performed to measure viability. Flow cytometric measurements were conducted on cells stained with CFDA/PI (carboxyfluorescein diacetate/propidium iodide) to evaluate the extent of cell damage. The same was performed on cells that were treated with PSL and RL alone.

Results: Separately, PSL and RL resulted in *L. monocytogenes* reduction of 0.4 and 0.1 log CFU/mL, respectively (p>0.05), with no more than 10% reduction in cell activity and no cell damage. PSL and RL together reduced *L. monocytogenes* by 6 log CFU/mL (p<0.05). The combination of PSL and RL has caused considerable damage to the cells. On average, 80% of cells had compromised cell membranes, 14% lost function, and only 6% remained active.

Significance: The investigation reveals a previously unappreciated property of PSL as a source of photosensitizers which are activated in cells by low doses of red light. This provides a new opportunity for treatment options in a variety of settings, including food decontamination.

P2-12 The Impact of Different Organic Acids on the Inactivation of *Listeria monocytogenes* on Food Matrices

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Introduction: *Listeria monocytogenes* is a challenging and persistent problem that might benefit from the optimization of different antimicrobials.

Purpose: Here, we aimed to compare the effect of citric acid (CA), malic acid (MA), and quinic acid (QA) against *L. monocytogenes* in culture and on food matrices in comparison to peroxyacetic acid (PAA).

Methods*:* The minimum inhibitory (MIC) and bactericidal (MBC) concentrations were assessed for each compound. Bacterial cultures were adjusted (OD_{600} of 0.05) and incubated with different concentrations of antimicrobials. OD_{600} was measured, and the MICs were determined as the lowest concentrations that showed no increase in OD_{600} after 24 h. For MBCs, cultures were serially diluted (10-fold), and aliquots were spread on nutrient agar. After incubation, the surviving colony forming units (CFU) were counted. The antimicrobials were also evaluated against artificially contaminated chicken breast meat and lettuce, which were selected as model food matrices. Each meat and lettuce sample was inoculated with ~5 log CFU/g of different *L. monocytogenes* strains and immersed in solutions containing the antimicrobials. CFU counts were determined as described earlier.

Results*:* Citric acid and malic acid had the lowest MICs (10 mg/ml) and MBCs (20 mg/ml) among the tested organic acids. MIC and MBC for quinic acid were 20 mg/mL and 20-40 mg/mL, respectively. Significant reductions in bacterial loads (≥ 2 log CFU/g) were observed with PAA ≥1,600 ppm and MA ≥160 mg/mL in chicken decontamination assays. While significant reductions in bacterial loads were observed with PAA ≥50 ppm, CA ≥80 mg/mL, and MA ≥40 mg/mL in lettuce decontamination assays.

Significance: The tested acids only impacted *L. monocytogenes* at relatively high concentrations. PAA outperformed the acids, showing a comparable or superior reduction in *L. monocytogenes* numbers at markedly lower concentrations. However, PAA did not sufficiently eliminate the bacterial pathogen, suggesting a need for more effective antimicrobials.

P2-13 The Impact of Coffee Extracts on the Control of Foodborne Bacterial Pathogens in Culture and on Different Food Matrices

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❖ ***Developing Scientist Entrant***

Introduction: Natural antimicrobials for controlling foodborne bacterial pathogens can be effective and desirable alternatives to commonly used chemicals. Coffee has been recognized for its bioactive ingredients that include antioxidants and antimicrobials.

Purpose: We aimed to evaluate the impact of coffee extracts, including citric acid (CA), malic acid (MA), and quinic acid (QA), as antimicrobials against *Campylobacter* spp., *Salmonella* serovars, and Shiga-toxin producing *Escherichia coli*.

Methods: The study involved assessing the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) for each of the coffee extracts. Bacterial cultures were adjusted (OD_{600} of 0.05) and then incubated with different concentrations of the extracts. For MBCs, cultures were also subjected to serial dilution (10-fold) and aliquots were spread on nutrient agar. After incubation, the surviving colony-forming units (CFU) were counted. The effectiveness of the coffee extracts was also evaluated against artificially contaminated chicken breast meat and lettuce. Each meat and lettuce sample were inoculated with approximately 5 log CFU/g of bacterial cocktails and immersed in solutions containing the extracts for 30 minutes. CFU counts were determined using the same procedure as described earlier.

Results: MICs for MA, CA, and QA ranged between 2.5 and 20 mg/mL and MBCs ranged between 5 and 40 mg/mL. Significant reductions in bacterial load (≥ 2 log CFU/g) were observed using ≥80 mg/mL MA and CA for *Campylobacter* on chicken samples. Significant reductions in *Salmonella* counts on chicken were noted using ≥80 mg/mL for MA and ≥160 mg/mL CA and QA. STEC numbers on chicken samples were significantly reduced with ≥160 mg/mL MA. *Salmonella* counts on lettuce were significantly reduced using ≥40 mg/mL of CA and MA, while 160 mg/mL MA was needed to significantly reduce STEC.

Significance: The evaluated extracts might not be optimal to control foodborne bacterial pathogens on meat and fresh produce.

P2-14 Prevalence and Comprehensive Characterization of *Campylobacter* Species Isolated from Poultry Meat in Retail Stores in Georgia, USA

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter* is a leading cause of foodborne gastroenteritis and is highly associated with the consumption of poultry products. In recent years, *Campylobacter* has been steadily developing resistance to first line drugs.

Purpose: Here, we aimed to determine the prevalence and molecular characteristics of *Campylobacter* in retail chicken breast meat in Georgia, USA.

Methods: Chicken breast samples (n=122) were obtained from major grocery stores. *Campylobacter* isolation was performed according to ISO-10272-2017 with modifications, and enriched samples were inoculated onto *Campylobacter* Blood-Free Selective Agar (mCCDA) with selective supplement. The plates were incubated under microaerobic conditions for 48 h at 37°C. Confirmation of identity and speciation were performed by PCR analysis. The isolates were also screened for the presence of eight pathogenic genes: *cdtA*, *cdtB*, *cdtC*, *cadF*, *flaA*, *virB*, *wlaN*, and *iam*. Whole-genome sequencing was performed to identify antibiotic resistance genes, clonal complexes, and sequence types (ST). Antibiotic resistance phenotypes were determined using the broth microdilution method.

Results: The prevalence of *Campylobacter* was 40.2% (49/122). *Campylobacter jejuni* was the predominant species, accounting for 85.7% of the isolates. The most prevalent *C. jejuni* CC was ST-353 CC (52.5%), followed by ST-48 CC (12.5%), while most *C. coli* isolates (77.8%) belonged to the ST-828 CC. *cdtABC* and *cadF* were detected in all *C. jejuni*, while *fla*A and *iam* were found in 85.7% and 34.7% of the isolates, respectively. Analysis using ResFinder v.4.4.2 database showed that 51% of the isolates carried β-lactamase encoding genes with bla_{OXA-61}, $bla_{OXA-193}$, $bla_{OXA-489}$ being the most prevalent, while 20.4% carried the *tet*(O) gene ang 8.2% had mutation in *gyrA*. Phenotypically, the highest resistance was observed against ampicillin (63.3%), followed by resistance to tetracycline (20.4%), nalidixic acid (18.4%) and ciprofloxacin (14.3%).

Significance: Our findings suggest that current processing interventions are insufficient to eliminate problematic *Campylobacter* strains on retailed chicken breasts.

P2-15 Evaluating Peracetic Acid Efficacy to Remove *E. coli* O157:H7 and *L. monocytogenes* Biofilms from Food-Contact Surfaces Using a Novel Bio-Inline® Reactor

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❖ ***Developing Scientist Entrant***

Introduction: Efficacy of bacterial biofilm removal by a chemical can be influenced by the surface topography, shear stresses, and chemical exposure time.

Purpose: The removal of biofilms formed by *E. coli* O157:H7 and *L. monocytogenes* were examined using Peracetic acid (PAA).

Methods: *E. coli* O157:H7 and *L. monocytogenes* biofilms were grown alone or with *R. insidiosa* on stainless steel (SS), PTFE, and EPDM in a CDC bioreactor at shear stresses 0.368 and 2.462 N/m² in 10% TSB for 48 hours. Coupons with biofilms were plugged-in to the Bio-inline® bioreactor and treated with 160 ppm PAA @ 1.2 liters/min for 1 and 4 minutes. Bacterial populations were quantified by spiral plating. Results from three separate replicates were analyzed for significant differences due to surface and exposure time. Material surface topography was recorded using a profilometer and scanning electron microscopy.

Results: For single species *L. monocytogenes* populations in biofilms grown at 2.462 N/m², reductions on PTFE (3.00 log CFU/cm²) were significant compared to EPDM (1.63 log CFU/cm²) after 4-minute exposure; similar trend was observed for single species *E. coli* O157:H7 biofilms. Significant reduction of *L. monocytogenes* in multispecies biofilms grown at 0.368 N/m² was observed on SS (1.46 log CFU/cm²) compared to EPDM (0.60 log CFU/cm²) after 1-minute exposure. Significant reductions were also observed on SS compared to PTFE after 4-min exposure. Multi-species *E. coli* O157:H7 biofilms grown at 2.462 N/m² and treated with PAA for 1-min resulted in significant reductions on SS (3.94 log CFU/cm²) compared to reductions on both PTFE (2.48 log CFU/cm²) and EPDM (1.11 log CFU/cm²). The surface roughness for PTFE and PC coupons were significantly higher than SS or EPDM; sharper peaks observed with PTFE.

Significance: SS is a suitable material compared to PTFE and EPDM for removing pathogens from single- and multi-species biofilms using PAA.

P2-16 Evaluation of a Novel Chlorine Dioxide Active Packaging System, Invisishield™, for Reduction of *Listeria monocytogenes* on Frozen Carrots

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Introduction: *Listeria monocytogenes* is a zero-tolerance adulterant in ready to eat (RTE) foods in the U.S. While not technically RTE, frozen vegetables are often treated as such by consumers and there is increasing concern about their potential contamination with *L. monocytogenes*. Chlorine dioxide (ClO2) is a promising antimicrobial with various food applications, one of those being controlled-release antimicrobial packaging systems.

Purpose: To evaluate a novel ClO_2-based antimicrobial packaging system for its efficacy against *L. monocytogenes* on frozen carrots.

Methods: Fresh carrots were diced, washed and blanched prior to inoculation with 10µl of an overnight culture of *L. monocytogenes*. Inoculated carrots were held at 4°C overnight prior to being mock-individually quick frozen (IQF) using liquid nitrogen. Inoculated IQF carrots were stored in glass jars containing InvisiShield™ packaging film with 0.29 g or 0.57g ClO_2 and held at -15°C±2°C for 5, 15 and 30 days. At each timepoint, carrots were collected and processed for enumeration of viable *L. monocytogenes* using Tryptic Soy and Modified Oxford Agars. Log reductions were calculated relative to respective inoculated controls (no ClO_2 treatment).

Results: There was no statistically significant difference in counts using the two agars ($p<0.05$), so data were pooled. Log reductions in *L. monocytogenes* on IQF carrots were 1.0±0.1, 1.3±0.6 and 1.2±0.6 at 5, 15 and 30 days for the 0.29g ClO_2 treatment, respectively. For the 0.57g ClO_2 treatment, log reductions were 1.2±0.5, 1.4±0.7 and 2.0±1.5 at 5, 15 and 30 days, respectively. The higher concentration with a 30-day exposure time outperformed the lower concentration at a 5-day exposure time ($p<0.05$).

Significance: This novel ClO2-based antimicrobial packaging system reduced the concentrations of *L. monocytogenes* on IQF carrots up to 2.0 log over 30 days frozen storage. Given the anticipated low *L. monocytogenes* counts in frozen vegetables, and the potential for post-thaw temperature abuse, this reduction is relevant to the industry and may provide public health benefit.

P2-17 Inhibition of *Listeria monocytogenes* in RTE Meats Using Cultured Celery Juice (VegStable® Secure)

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Introduction: Ready-To-Eat (RTE) meats pose a risk for harboring pathogenic bacteria due to their long storage and high water activity. Tradtionally, this has been controlled by the addition of organic acid salts into the meat formulation. Consumer interest in natural ingredients has made synthetic antimicrobials unattractive for labelling. Culturing vegetable juice to be high in antimicrobial acids presents a clean label alternative to synthetic ingredients.

Purpose: Determine the antimicrobial efficacy of cultured celery juice against *Listeria monocytogenes* in cured and uncured meat models at 4°C for up to 120 days.

Methods: A variety of meat models were formulated with 3.0-4.2% cultured celery juice, cooked, cooled, and inoculated with a cocktail of *L. monocytogenes*. The meats were stored at 4°C and the *L. monocytogenes* cell count was monitored for the expected shelf-life using individual vacuum- sealed packages in duplicate. The cell count was compared to a negative control containing no antimicrobial and a positive control containing 3% potassium lactate-sodium diacetate. The meat matrices tested included: formed ham, uncured turkey roll, and beef hot dogs.

Results: Cultured celery juice used at 3.8% effectively prevented the outgrowth of *L. monocytogenes* in formed ham that included nitrite from celery powder for the study duration of 120 days. In ham containing nitrite alone, *L. monocytogenes* outgrowth was observed at 60 days. In an uncured deli turkey model 3.8% cultured celery juice prevented the outgrowth of *L. monocytogenes* for 90 days. In hot dogs, 3.4% cultured celery juice was found to prevent *L. monocytogenes* outgrowth at a lower active concentration than a synthetic control for 90 days.

Significance: Cultured celery juice rich in organic acids as a novel antimicrobial ingredient used in cured and uncured RTE meats to control *L. monocytogenes* growth.

P2-18 A Novel, Clean-Label Antimicrobial Solution to Control the Outgrowth of *Listeria monocytogenes* in Deli-style Turkey

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Introduction: *Listeria monocytogenes* is a hurdle for ready-to-eat food products, especially deli meats. In the past, plant ingredients have been effective in controlling the outgrowth of microorganisms in various food products. A novel, cultured spice antimicrobial was developed as an alternative to current antimicrobials to suppress the outgrowth of *L. monocytogenes* in ready-to-eat foods.

Objective: To evaluate the efficacy of a novel cultured spice prototype in controlling the outgrowth of *L. monocytogenes* in deli-style turkey.

Methods: Deli-style turkey prepared without antimicrobial (NC), 1.4% Prototype Cultured Spice (RCE-1), or 2.0% Prototype Cultured Spice (RCE-2) was inoculated with a 5-strain cocktail of *L. monocytogenes* to achieve a population of ca. 3.0 log CFU/g. All samples were vacuum-packaged and stored at 4.4°C/40°F until enumeration. Samples were diluted with buffered peptone in a 1:2 ratio and stomached at 230 rpm for 30 s. The *L. monocytogenes* inoculated samples were spread-plated on Modified Oxford agar (37°C for 48 h). Non-inoculated samples were prepared and spread-plated on de Man, Rogosa, and Sharpe agar (30°C for 48 h) to ensure no background interference with *L. monocytogenes* control.

Results: The *L. monocytogenes* starting population was ca. 2.95 log CFU/g. In the NC sample, the *L. monocytogenes* population reached 1-log and 2-log outgrowth after 6 and 7 d, respectively. The samples containing RCE-1 and RCE-2 were enumerated until day 150 and never reached 1-log outgrowth. The treatments saw significantly ($p<0.05$) lower *L. monocytogenes* populations than the NC samples. The LAB population in the non-inoculated samples remained below the detection limit (<0.30 log CFU/g) throughout the entire shelf life. This indicates that the addition of the novel antimicrobial was the leading cause of *L. monocytogenes* inhibition.

Significance: The developed novel cultured spice ingredient serves as an effective antimicrobial in controlling *L. monocytogenes* in deli turkey applications.

P2-19 Buffered Vinegar Alternatives for Controlling the Outgrowth of *Listeria monocytogenes* in Uncured Deli-style Meat

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Introduction: Buffered vinegar has been shown to inhibit the outgrowth of *L. monocytogenes* in refrigerated meat products. However, the new rules stated by the Canadian Food Inspection Agency require that the products containing buffered vinegar must be labeled with the associated buffering agent.

Purpose: To validate buffered vinegar alternatives for inhibiting the outgrowth of *L. monocytogenes* in deli-style turkey.

Methods: Deli-style turkey samples prepared with four formulations (no antimicrobial, 0.75% Verdad® Powder N30 + 2% Verdad® N16 (CS-1), 1.3% Verdad® Powder N38 (CS-2), and 1.9% Verdad® Powder N38 (CS-3)) were inoculated with a 5-strain cocktail of *L. monocytogenes* at ca. 3.0 log CFU/g or left uninoculated, vacuum-packaged, and stored at 4.4°C/40°F. For enumeration, samples were transferred to a sterile stomacher bag to which the required amount of buffered peptone water was added to achieve a 1:2 dilution. The diluted sample was stomached and spread-plated onto Modified Oxford agar (37°C for 48 h) to enumerate *L. monocytogenes* and the uninoculated samples were spread-plated onto de Man, Rogosa, and Sharpe agar (30°C for 48 h) to enumerate lactic acid bacteria.

Results: Samples were analyzed for 1-log and 2-log outgrowth of *Listeria* for 147 days. The sample containing no antimicrobial realized 1-log and 2-log *L. monocytogenes* outgrowth on days 7 and 12, respectively. The samples containing CS-2 exceeded 1-log and 2-log *L. monocytogenes* outgrowth on days 56 and 77, respectively. Samples prepared with CS-1 and CS-3 did not exceed 1-log *L. monocytogenes* and remained bacteriostatic. The growth rate of *L. monocytogenes* in the negative control sample was significantly ($p<0.05$) higher than the sample containing CS-2 treatment. The background flora was below the detection limit (<0.30 log CFU/g) throughout the shelf-life period.

Significance: The tested treatments (CS-1 and CS-3) were effective in extending the shelf-life of deli-style turkey and can be used as a buffered vinegar alternative.

P2-20 A Novel Buffered Lactic Acid Solution as a Surface Treatment for *Salmonella* spp. Reduction in Fresh Pork

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Introduction: *Salmonella* is a Gram-negative, non-spore forming pathogen that is considered a significant risk in pork. Despite numerous interventions throughout the processing, *Salmonella* continues to proliferate in pork leading to recalls and illnesses.

Objective: To evaluate the efficacy of a novel buffered lactic acid solution (Purac® BF S/210) on the surface of fresh pork for *Salmonella* reduction.

Methods: Fresh pork ham pieces were portioned into 10 x 10 cm cubes. Each pork cube was inoculated with a 5-strain *Salmonella* cocktail at a population of ca. 4.0 log CFU/cm^2. The inoculated samples were placed in the biosafety safety cabinet for 30 min for bacterial attachment. Samples for one treatment were left untreated (UT), while samples from the other two treatments (Distilled water (W) and Purac® BF S/210 (BFS)) were sprayed using a pressurized spray system set to 20 psi to deliver approximately 1 mL of solution per cm^2 of the sample. For enumeration, the samples were diluted with Dey Engley neutralizing buffer, stomached, and spread-plated on Xylose Lysine Tergitol-4 agar (37°C for 24±2 h). Two replications of the study were conducted. Minitab 20.2 was used to perform Tukey's test to determine any significant difference between the treatments at $\alpha = 0.05$.

Results: The *Salmonella* population in the UT samples had a survival of 4.09 log CFU/cm^2, while W-treated samples had a survival of 3.75 CFU/cm^2. Samples treated with BFS had significantly ($p<0.05$) lower *Salmonella* population of 3.08 CFU/cm^2. Overall, the application of BFS resulted in a 1.01 and 0.67 log *Salmonella* reduction compared to the UT and W treatments.

Significance: The surface application of buffered lactic acid solution resulted in a one-log reduction of *Salmonella* on the surface of fresh pork. This study validates the efficacy of a novel buffered lactic acid solution as a processing aid for the fresh meat industry.

P2-21 Inhibition of *Clostridium perfringens* Spores by Antimicrobial Ingredients during Extended Cooling of Cooked Uncured Meat

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Introduction: *Clostridium perfringens* is a concern in cooked uncured meats that are cooled slowly, allowing the surviving bacterial spores to germinate, outgrow, and produce toxins. The FSIS stabilization guideline (Appendix B) defines cooling limits for uncured meat and poultry products to inhibit *C. perfringens* outgrowth. However, cooling process deviations are common in the meat and poultry processing industry.

Objective: To validate the efficacy of various antimicrobials to inhibit the *C. perfringens* outgrowth during extended cooling of uncured meat.

Methods: Ground turkey formulated with five treatments (no antimicrobial, 2.14% *Opti.Form*® PD4 (V1), 0.75% Verdad® Powder N6 (V2), 1% Verdad® Powder N30 (V3), and 1.5% Verdad® Opti Powder N510 (V4)) were inoculated with a 4-strain *C. perfringens* cocktail at ca. 3.0 log CFU/g. Samples (25 g) were vacuum-packaged, flattened to uniform thickness, and cooked at 75°C for 20 min. The cooked samples were cooled to 54.4°C and transferred to a programmable water bath for dynamic cooling. Samples were cooled from 54.4 to 26.7°C in either 1.5, 2, or 5 h, then from 26.7 to 4.4°C in either 5, 8, or 10 h (D1: 6.5 h, D2: 10 h, and D3: 15 h). At each pull point, three samples were removed and spread-plated on Tryptose Sulfite Cycloserine agar (37°C for 24±2 h) to estimate *C. perfringens* outgrowth.

Results: The samples had an average moisture of 74.51±0.14%, pH 6.35±0.01, and water activity 0.981±0.001. *C. perfringens* population increased by 0.60 and 3.14 log in meat without antimicrobials during D2 and D3 cooling, respectively. *C. perfringens* outgrowth (<1 log) was inhibited in samples containing antimicrobials for all cooling profiles. Samples containing V4 had a significant ($p<0.05$) reduction in *C. perfringens* population (0.36-1.03 log) as the cooling time increased from 6.5 h to 15 h.

Significance: The application of antimicrobial ingredients effectively inhibited *C. perfringens* outgrowth in uncured meat during extended cooling.

P2-22 Comparing Efficacy of Vinegar-Based Antimicrobials in Inhibiting the Outgrowth of *Listeria monocytogenes* and Extending the Shelf-Life of Ready-to-Eat Meat and Poultry Products

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Introduction: Listeria monocytogenes is one of the important food-borne pathogens that can grow at refrigeration temperature and even in low oxygen environment like vacuum packaged products. Buffered vinegar and cultured dextrose are commonly used natural antimicrobials for food applications. In our past studies buffered vinegar had been proven to inhibit outgrowth of L. monocytogenes and extending the shelf-life of RTE food products.

Purpose: To compare the antimicrobial efficacy of buffered vinegar-cultured dextrose blend, concentrated buffered vinegar and buffered vinegar in inhibiting the outgrowth of *Listeria monocytogenes* and extending the shelf-life of RTE turkey and roast beef.

Method: Slices of RTE turkey and roast beef were formulated with buffered vinegar and cultured dextrose blend (T1) at 1.0%, concentrated buffered vinegar (T2) at 0.6%, and buffered vinegar (T3) at 0.75% usage rates were inoculated with cold-adapted five-strain cocktail of *Listeria monocytogenes* (ATCC 19111, 19112, 19115, 19118, 13932) at a targeted inoculum rate of 2-3 log CFU/g. After inoculum attachment, samples were vacuum packaged and stored at 39 ±1.5 °F. A set of samples subjected to the same packaging and storage conditions were tested for shelf-life. Samples were enumerated for *L. monocytogenes* using FDA-BAM method; total aerobic bacteria, lactic acid bacteria, yeast, and mold using respective AOAC standard methods.

Results: No significant difference was found between the treatments T1 and T3 ($p>0.30$) in inhibiting the growth of *Listeria monocytogenes* in turkey and roast beef. Treatment T2 was not effectively inhibiting the growth of *L. monocytogenes* in both the tested food matrices compared to T1 and T3 ($p<0.05$). Microbial counts for shelf-life testing remained low under 6 log CFU/g for up to 75 days.

Significance: Buffered vinegar and blend of buffered vinegar-cultured dextrose were equally effective in controlling the growth of L. monocytogenes in turkey and roast beef.

P2-23 Efficacy of Liquid Acetate-Diacetate Blend in Inhibiting the Outgrowth of *Listeria monocytogenes* in Hotdogs

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Introduction: *Listeria monocytogenes* is a facultative, gram-positive bacteria, persists in various food production environments and causes potential threat for public health. Application of antimicrobial blends such as lactate-acetate and lactate-diacetate in food have been studied widely for anti-listerial properties. The objective of this study was to determine the efficacy of liquid acetate-diacetate blend in inhibiting the outgrowth of *Listeria monocytogenes*

in ready-to-eat (RTE) foods.

Purpose: To determine the efficacy of liquid acetate-diacetate blend in inhibiting the outgrowth of *Listeria monocytogenes* and extending the shelf-life of hotdogs.

Method: Hotdog samples with lactate-diacetate – a positive control (1.14%) and treatments with liquid acetate-diacetate blend (0.6%, 0.8%, and 1%) were surface inoculated with a five- strain (ATCC 19111, 19112, 19115, 19118, 13932) cold-adapted cocktail of *Listeria monocytogenes* at a target inoculum rate of 2-3 log CFU/g. Samples were vacuum packaged and stored at 39 ±1.5°F. Samples were enumerated for *L. monocytogenes* using the FDA-BAM method. Shelf-life testing was performed on uninoculated samples, stored at 39 ±1.5 °F, for Total Aerobic, Lactic Acid Bacteria and Yeast & Mold Counts using respective AOAC standard methods.

Results: Liquid acetate-diacetate blend at various usage rates showed significant difference in controlling *Listeria monocytogenes* compared to lactate-diacetate with $p<0.05$ (*t*- test) for up to 70 days. Blend at 0.6% and 0.8% usage rates showed no significant difference in controlling *Listeria monocytogenes* ($p>0.10$). Blend at 1.0% usage rate significantly inhibited the growth of *Listeria monocytogenes* with $p<0.05$ (*t*- test) compared to 0.6% and 0.8% usage for up to 110 days. Microbial counts for shelf-life testing remained low under 6 log CFU/g as well.

Significance: Liquid acetate-diacetate blend at 1.0% usage rate was effective in controlling the growth of *Listeria monocytogenes* and increasing the shelf-life of hotdogs.

P2-24 Comparing the Efficacy of Buffered Vinegar and Cultured Dextrose in Pork Sausages in Controlling Overgrowth of *Leuconostoc mesenteroides*

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Introduction: Food security and sustainability have received considerable attention from the food industry to prevent food spoilage. For food to remain edible and nutritious before microbial spoilage occurs is dependent on the storage temperature, moisture, and other factors such as water activity and pH. Spoilage typically occurs due to overgrowth of microbes such as lactic acid bacteria (LAB) and aerobic bacteria. *Leuconostoc mesenteroides* is one such LAB that has been reported to cause food spoilage in some types of food matrices.

Purpose: To compare the efficacy of buffered vinegar and cultured dextrose in controlling the growth of *Leuconostoc mesenteroides in* pork sausages.

Method: A negative control (T1), 0.7% buffered vinegar (T2) and 1% cultured dextrose (T3) treated samples of pork sausages were inoculated with *Leuconostoc mesenteroides* isolated from a spoiled pork-based product to the target inoculum level of 3-4 log CFU/g. Samples were vacuum packaged and stored at 39 ±1.5°F. De Man-Rogosa-Sharpe agar petri plates were used for enumeration, incubating at 35±1°C for 48h under microaerophilic conditions. Uninoculated, vacuum packaged samples, stored at 39 ±1.5 °F, were used for shelf-life testing of Total Aerobic Count, Lactic Acid Bacteria and Yeast and Mold Count at each sampling point using standard respective AOAC methods.

Results: *t*-test showed no significant difference in controlling *Leuconostoc mesenteroides* for up to 61 days between treatments T1 and T3 ($p> 0.10$). However, both T1 and T3 were significantly different from the treatment T2 with $p<0.05$. Also, T2 showed better control throughout the study than T3. Shelf-life indicators remained low under 6 log CFU/g for up to 61 days in all the samples.

Significance: Buffered vinegar was effective in controlling the growth of *Leuconostoc mesenteroides* in pork sausages and can be used to prevent spoilage due to growth of lactic acid bacteria in pork sausages.

P2-25 The Effect of Vinegar and Conventional Acetate-Based Preservatives on Outgrowth of *Listeria monocytogenes* in Pork Ham

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Introduction: Sodium- and potassium-neutralized vinegar and acetates can be used to reduce food safety risks and improve shelf life by reducing outgrowth of pathogens and other microorganisms in meat products.

Purpose: Reducing food safety risks by decreasing outgrowth of *Listeria monocytogenes* in pork ham by incorporating clean-label and conventional acetate-based preservatives.

Methods: Five treatments of pork ham (80% pork meat, 15.5% water, 2% starch, 1.4% salt, 0.4% phosphate and 100 ppm nitrite) included a control without preservatives and 0.7-0.75% of IsoAge DV 105, IsoAge DV 303, Provian K and Provian N. The sliced ham was inoculated with a five-strain *Listeria monocytogenes* inoculum by surface inoculation, targeting 2-3 log CFU/g. Slices of ham were vacuum-packed in sterile plastic bags and stored at 4°C and 15°C (accelerated study) for 120 and 21 days respectively. At each sampling point, samples were homogenized and plated onto PALCAM agar. Treatments were compared using one-way ANOVA ($p<0.05$).

Results: At 4°C, control samples without preservatives reached a stationary level of 8.6±0.1 log CFU/g after 29 days, while all samples containing vinegar and conventional preservatives did not reach the spoilage threshold of 2 log CFU/g outgrowth within the storage period of 120 days. To determine differences between the treatments, an accelerated test at 15°C was executed, where the control samples reached a stationary level of 9.4±0.1 log CFU/g in 8 days. Sodium-buffered products show slight improved performance compared to potassium-buffered products for both clean-label (7.4±0.3 and 8.3±0.2 log CFU/g) respectively after 13 days) and conventional products (7.5±0.1 and 7.9±0.1 log CFU/g) respectively after 13 days), due to a higher acetate content in the final product.

Significance: The results of this study demonstrate the possibility to use clean-label and conventional acetate-based preservatives to reduce food safety risks of pork ham.

P2-26 The Effect of Acetate-Based Preservatives on Outgrowth of *Listeria monocytogenes* and *Pseudomonas* sp. in Plant-Based Chicken Chunks

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Introduction: Refrigerated plant protein-based foods are susceptible to similar food safety issues as meat, such as *Listeria monocytogenes*, an opportunistic pathogen with a relatively high mortality rate.

Purpose: Reducing food safety risks due to outgrowth of *Listeria monocytogenes* in vegetarian chicken chunks by incorporating a clean-label acetate-based preservative (prototype SB).

Methods: Vegetarian chicken chunks were prepared containing 0.5% and 0.75% prototype SB and a control without antimicrobials. The vegetarian chicken chunks were inoculated with a five-strain *Listeria monocytogenes* inoculum, targeting 2-3 log CFU/g and vacuum-packed in portions of approximately 15 g in sterile plastic bags and stored at 7°C for 17 days, followed by 18 days at 10°C, resembling the storage conditions during retail and at the consumer. Additionally, portions of non-inoculated vegetarian chicken chunks were incubated at the same temperature profile for enumeration of *Pseudomonas* sp. At each sampling point, samples were homogenized using a stomacher and plated onto PALCAM (*Listeria* sp.) and *Pseudomonas* agar. Treatments were compared using one-way ANOVA ($p<0.05$).

Results: The control treatment inoculated with *Listeria monocytogenes* showed the fastest outgrowth, reaching the stationary phase of 8 log CFU/g after 15 days. 0.5% prototype SB showed significantly ($p<0.05$) less outgrowth, reaching only 4.2±0.9 log CFU/g after 35 days, whereas 0.75% prototype SB remained at inoculation level of 2.5±0.2 log CFU/g during 35 days at these abusive temperature storage conditions. *Pseudomonas* sp. showed a similar trend for the different treatments, with the control reaching 8.2±0.2 log CFU/g within 15 days, 0.5% prototype SB reaching 5.6±0.2 log CFU/g after 35 days and 0.75% prototype SB remaining at approximately initial microbial load of 2.0±0.2 log CFU/g for 35 days.

Significance: The results of this study demonstrate the possibility to use clean-label acetate-based preservatives to increase shelf life and reduce food

safety risks of plant-based chicken chunks.

P2-27 The Effect of Nourishield D4010 on Outgrowth of *Listeria monocytogenes* and Slime-Forming *Leuconostoc mesenteroides* in Frankfurters

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Introduction: Plant extracts and vinegar can be implemented in new clean-label multifunctional systems for food preservation, by reducing microbial growth and improving oxidative stability of food products.

Purpose: Reducing food safety risks and food spoilage due to outgrowth of *Listeria monocytogenes* and *Leuconostoc mesenteroides* in frankfurters using Nourishield D4010, a clean-label multifunctional system consisting of vinegar and a plant extract.

Methods: Five treatments of frankfurters were prepared, including a control without preservatives and samples containing 0.5% and 0.75% Provian NDVs and 0.5% and 0.75% Nourishield D4010. Each treatment was divided into two portions, of which one was inoculated with a five-strain *Listeria monocytogenes* inoculum (serotypes 1-2a, 1-2c, 4a, 4b, 6a/6b) and the other was inoculated with a two-strain slime-producing *Leuconostoc mesenteroides* inoculum, targeting 2-3 log (CFU/g). The meat was packed in portions of approximately 15 g in sterile plastic bags and stored at both 4°C and 7°C for 45 days (*Leuconostoc*) and 130 days (*Listeria*). At each sampling point, samples were homogenized and plated onto PALCAM (*Listeria*) and MRS (*Leuconostoc*) agar.

Results: For both *Listeria* and *Leuconostoc* at 0.5% and 0.75%, Nourishield D4010 shows significantly ($P<0.05$) improved antimicrobial performance compared to Provian NDVs. For *Listeria monocytogenes*, 0.5% Nourishield D4010 performs better (4°C) or equal (7°C) to 0.75% Provian NDVs. At 4°C, 2 logs outgrowth of *Listeria* was reached after 41 and 75 days for 0.75% NDVs and 0.5% Nourishield D4010 respectively. At 7°C, 2 logs outgrowth of *Listeria* was reached after 22 days for both 0.75% NDVs and 0.5% Nourishield D4010. Additionally, in samples inoculated with *Leuconostoc*, visually less slime formation was observed in Nourishield D4010 samples compared to NDVs and control samples.

Significance: The results of this study demonstrate the possibility to increase food safety and shelf life of frankfurters using a clean-label multifunctional system of a plant extract and vinegar, Nourishield D4010.

P2-28 The Effect of Vinegar and Conventional Acetate-Based Preservatives in Raw Beef Burgers

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Introduction: Raw beef burgers are widely consumed meat products but have a limited shelf life due to a high initial microbial load and favorable composition for microbial growth.

Purpose: Reducing food safety risks and increasing shelf life by decreasing outgrowth of pathogens and other microorganisms in raw beef burgers by incorporating clean-label and conventional acetate-based preservatives.

Methods: Five treatments of beef burgers (91% beef trim, 7.55-7.6% water, 0.65% salt) included a control without preservatives and 0.7-0.75% of IsoAge DV 105, IsoAge DV 303, Provian K and Provian N. The raw beef burgers were vacuum-packed in portions of approximately 20 g in sterile plastic bags and stored at 4°C for 24 days. At each sampling point, samples were homogenized using a stomacher and plated onto TSA, MRS, VRBG and selective *Pseudomonas* agar to obtain numbers for total plate count, lactic acid bacteria, Enterobacteriaceae and *Pseudomonas* sp. respectively. Treatments were compared using one-way ANOVA ($p<0.05$).

Results: Raw beef burgers without preservatives surpassed 8.5±0.2 log CFU/g within 10 days of storage for both total plate count and lactic acid bacteria, whereas all samples containing preservatives showed significant ($p<0.05$) reduction in outgrowth. Additionally, all samples containing preservatives prevent outgrowth of Enterobacteriaceae and *Pseudomonas* sp. during 24 days whereas the control samples reach 7.0±0.3 log CFU/g within 13 days for both organisms.

Significance: The results of this study demonstrate the possibility to use clean-label and conventional acetate-based preservatives to increase shelf life and reduce food safety risks of raw beef burgers.

P2-29 Efficacy of Dried or Liquid Vinegar and Lemon Buffered Vinegar Systems against *Listeria monocytogenes*

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Introduction: Buffered vinegar and natural organic acids qualify for clean label solutions and provide benefits such as flavor, shelf-life extension, and food safety impact.

Purpose: To assess the minimum inhibitory concentrations (MIC) against *Listeria monocytogenes* for clean-label solutions such as liquid buffered vinegar systems (IsoAge 210L, BV1), (IsoAge 240L, BV2), lemon buffered vinegar (Nourishield 5000L, BV3) and dry buffered vinegar system (IsoAge DV100, BV4).

Methods: Samples of BV1, BV2, BV3 and BV4 were prepared at varying concentrations (0-2%) in Brain Heart Infusion broth (BHI). Samples were prepared at two pH levels: 5.8 and 6.0. Samples were aliquoted into 100-well plates then inoculated with a cocktail of *Listeria monocytogenes* strains (serotype 1/2a, 1/2b, 4a and 4b). The OD was measured at 600 nm using Bioscreen C, incubated at 30°C. Growth curves were fitted using modified Gompertz equation to determine the maximum growth rate (μmax; h^{-1}). The MIC was defined as the lowest concentration at which no growth occurred. Data was analyzed using one-way ANOVA ($p<0.05$).

Results: All solutions tested exhibited a similar inhibition pattern. There was no significant difference in the MIC of solutions at pH 5.8 and 6.0 except for BV3 ($p<0.05$). The most effective solution was BV4 with an MIC of 1.7% and 1.8% respectively. BV1 and BV2 performed similarly with an MIC of 2.3% and 2.2% (pH 5.8) and 2.8% and 2.5% (pH 6) respectively. Finally, BV3 exhibited the highest MIC of 3.3% and 4.3% at pH 5.8 and 6.0 respectively.

Significance: The study highlights the antimicrobial efficacy of clean label buffered vinegar systems against food safety related meat microorganisms and provides industry with a natural ingredient.

P2-30 Evaluation of *Salmonella* Typhimurium Inhibition by Vinegar and Natural Flavor

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Introduction: Vinegar and natural flavor are clean label friendly ingredients that are known for their antimicrobial properties. Combined use could have potential to be a robust multi-functional antimicrobial system.

Purpose: To assess the inhibitory effect of vinegar (Innovation concept; IC1 & IC2) and natural flavor (IC3, IC4, IC5) against *Salmonella* Typhimurium.

Methods: Solutions were prepared in Brain Heart Infusion broth (BHI) at pH 6.0 at varying concentrations of IC1 (0.5-0.75%), IC2 (0.5-0.75%), IC3 (0.25-0.5%), IC4 (0.25-4%), and IC5 (0.25-4%). Samples were prepared in the same manner with combinations of 0.75% IC1 with each natural flavor (0.25-1%). Samples were aliquoted into 100-well plates then inoculated with *Salmonella* typhimurium. The OD was measured at 600 nm using Bioscreen C, incubated at 30°C. Growth curves were fitted using modified Gompertz equation to determine the maximum growth rate and lag time ($\mu max h^{-1}$;h). The MIC was defined as the lowest concentration at which no growth occurred. Data was analyzed using one-way ANOVA ($p<0.05$).

Results: The most effective solution was IC1 with MIC at 0.5%. Similar results of no growth of *Salmonella* typhimurium were observed in samples of 0.5% IC1 with each natural flavor. All other solutions had significantly ($p<0.05$) reduced the growth rate and increased lag time of *Salmonella* compared to the control (7.85 h^{-1}; 0.17 h). Natural flavor in combination with IC2 exhibited a synergistic inhibitory effect. IC2 at 0.75% (1.44 h^{-1};0.23 h) was most effective when tested in combination with 1.5% IC4 (0.40 h^{-1};0.47 h).

Poster

Significance: The study demonstrates the antimicrobial efficacy of vinegar-natural flavor and natural flavor as well the benefits of multi-functional preservation system for food safety.

P2-31 Impact of Sodium Chloride Concentrations on Antilisterial Efficacy of Potassium-Based Organic Acid Salts and Fermentates

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Introduction: Canadian mandate for front-of-pack declaration by 2026 are encouraging processors to consider sodium-reduced formulas. Traditionally sodium lactate/di-acetates, used as *Listeria monocytogenes* control could be replaced by potassium based organic acid salts lowering sodium in meat products.

Purpose: To assess the antilisterial efficacy of potassium acetate/diacetate (Provian K) and potassium acetate/ferment peptide (Nourishield 2000L) at varying sodium chloride concentrations.

Methods: Varying concentrations of Provian K (0.5, 0.75%), Nourishield 2000L (2%, 3%) and control were prepared in Brain Heart Infusion broth with either high-sodium (970mg/100g) or low-sodium (355mg/75g) or no-sodium formulas. Samples, adjusted to pH 6.0, was aliquoted and inoculated with *L. monocytogenes* (serotype 1/2a, 1/2b, 4a, 4b) into 100-well plates and incubated at 30°C using Bioscreen-C. Growth curves (600 nm) were fitted using modified Gompertz equation to determine the maximum growth rate and lag time (μmax; h^{-1};h). Differences among the treatments were determined using one-way ANOVA with significance set at $P< 0.05$.

Results: All the products tested at low/high salt concentration, exhibited similar inhibition pattern and showed significantly ($P<0.05$) reduced growth rate and increased lag time (0.10-0.54 h^{-1}; 2-6 h) compared to control (0.93-1.13 h^{-1}; 0.9-1h). Antilisterial performance from most effective to least was 3%, 2% Nourishield 2000L and 0.75%, 0.5% Provian K. For both low/high-sodium concentrations, samples with 2% or 3% Nourishield 2000L exhibited no growth and increased lag time (6 h) for *L. monocytogenes* serotype 1/2a whereas for other serotypes, 3% showed a reduced growth rate and increased lag time (μmax 0.10-0.20 h^{-1};3-5 h) compared to 2% (0.26-0.32 h^{-1};2.3-2.8 h). Similar trends were observed for Provian K, for *L. monocytogenes* serotype 1/2a, they exhibited reduced growth rate and increased lag time (0.18-0.15 h^{-1};3-6 h) compared to other serotypes (0.31-0.54 h^{-1}; 2 h).

Significance: Potassium based organic acid salts could help reduce sodium in meat and food formulations without comprising food safety.

P2-32 Sanitizer Susceptibility of Leaf-Associated *Escherichia coli* O157:H7 during Washing of Inoculated Romaine Lettuce after Simulated Source or Forward Processing Conditions

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Introduction: Lettuce is processed locally (source processing, SP) or remotely after long-haul transportation (forward processing, FP) but the effects of processing on *Escherichia coli* O157:H7 sanitizer susceptibility during lettuce washing is unknown.

Purpose*:* To evaluate sanitizer susceptibility of lettuce-associated *E. coli* O157:H7 during washing of lettuce subjected to simulated source and forward processing.

Methods*: E. coli* O157:H7 outbreak strain 2705C was spot-inoculated on leaves of commercially grown Romaine. Leaves underwent simulated source and forward processing, then cut into strips and washed in free chlorine (Cl), peroxyacetic acid (PAA) or DI-water for 1 min. Bacteria were recovered for plate counting within 1 h or after 7 days of storage at 3°C on MacConkey (MAC), with or without Tryptic Soy agar overlay. Injury was estimated by subtracting MAC counts from TSA-overlay counts. Quantitative-PCR amplification of gene *stx2* after propidium monoazide treatment was used to detect the level of viable but not culturable (VBNC) cells, determined by subtracting the VBNC population from TSA-overlay counts. Data were analyzed with multiple regression.

Results*: E. coli* 2705C populations on lettuce were affected by lettuce processing conditions, sanitizer and storage ($p<0.005$). Slightly higher 2705C counts were recovered from SP lettuce (5.0 log CFU/g) after washing than FP lettuce (4.8 log CFU/g, $p<0.05$). Injury level did not differ by processing condition, but VBNC cell estimates from FP lettuce (0.6 log) were higher than SP (0.4 log, $p<0.05$). Higher counts were measured on lettuce after 50 ppm Cl wash (4.5 log CFU/g) than 80 ppm PAA wash (4.0 log CFU/g, $p<0.01$), and VBNC cell estimates were lower ($p<0.001$). No difference in injury was detected. *E. coli* 2705C counts from FP lettuce washed in 50 ppm Cl decreased ~1.1 log in storage ($p<0.05$), without affecting injury or VBNC cells.

Significance: *E. coli* O157:H7 associated with Romaine previously subjected to FP exhibited enhanced susceptibility to various sanitizers during lettuce washing, and populations declined further in storage.

P2-33 Luteolin Exhibits Antibiofilm and Antibacterial Actions against *Salmonella* Typhimurium and *Escherichia coli* by Impairing Cell Adhesion, Membrane Integrity, and Energy Metabolism

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Introduction: The persistence of foodborne pathogens, such as *Salmonella Typhimurium and Escherichia coli*, in food processing and medical facilities often leads to severe disease outbreaks and the emergence of antimicrobial resistance. Combatting these challenges, global interest in phytochemicals, including luteolin (3',4',5,7-tetrahydroxyflavone), for advanced antimicrobial research is actively growing because of its unique features to exhibit potent antimicrobial actions by effectively interacting with cell membranes and targeting many critical functional sites of pathogens.

Purpose: As the antimicrobial activities of luteolin remain mostly elusive, the purposes of this study were to evaluate its antibiofilm and antibacterial potential against *S*. Typhimurium and *E. coli*, along with understanding associated mechanistic actions, encompassing effects on cell adhesion, membrane integrity, and energy production.

Methods: The antibiofilm efficacy of luteolin was evaluated against *S*. Typhimurium and *E. coli* using eggshell, stainless steel, and silicon rubber surfaces. As part of antibiofilm biomechanics, anti-adhesion effects were explored by tracking time-dependent cell adhesion (4-12h) on silicon rubber surfaces and assessing alterations in cell surface hydrophobicity (4-24h). As part of antibacterial biomechanics, this study further evaluated its membrane-disrupting actions by assessing ß-galactosidase activities (1-4 h) and adverse effects on energy production by ATP quantification (4-24h) assay. Moreover, the CLSM and TEM analyses were also performed to illustrate its bactericidal effects on biofilm and planktonic populations, respectively.

Results: Findings evidenced that luteolin remarkably prevented pathogenic biofilm formation on biotic and abiotic surfaces by resisting irreversible cell adhesion and interfering with hydrophobic interactions between cells and contact surfaces. Our comprehensive biochemical and visual analysis revealed that luteolin could increase membrane permeability and severely damage membrane integrity, causing cell lysis. Simultaneously, luteolin also impaired the balance in cellular ATP metabolism.

Significance: This study offers valuable insights into future research for developing membrane-targeting novel antimicrobials to combat the persistence and antimicrobial resistance of foodborne pathogens.

P2-34 Development of Poly Lactic Acid-Based Natural Antimicrobial Film with Caprylic Acid against *Salmonella* Biofilm Contamination in Meat Industry

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Introduction: *Salmonella* is one of the prevalent pathogenic bacteria in meat industry causing significant economic loss by forming biofilm on various food contact surfaces, leading to cross-contamination of final products. Recently, antimicrobial active packaging is getting attention to reduce the risk of microbial contamination during distribution and storage process of meat.

Purpose: The purpose of this study is to develop antimicrobial poly-lactic acid (PLA) film with incorporation of caprylic acid (CA) and evaluate its changes in physico-chemical properties obtained by addition of CA.

Methods: The minimum inhibitory concentration (MIC) of CA was confirmed following by the micro-broth dilution method. After that, CA was applied to PLA film using solvent-casting method. The biofilm inhibitory effect and anti-microbial efficacy of CA-PLA film against *S*. Typhimurium and *S*. Enteritidis, intermolecular interactions and barrier properties of CA-PLA film were evaluated by comparing PLA film and CA-PLA film.

Results: The biofilm formation of *Salmonella* was reduced to the level of under detection limit (< 1 log CFU/cm^2) by 4.8 % CA-PLA film. The highest reduction values of 2.58 log CFU/ml for *S*. Typhimurium and 2.59 log CFU/g for *S*. Enteritidis were achieved on chicken breast at 25 °C for overnight with 4.8 % CA-PLA film, without any quality (color and texture) losses. The presence of CA in CA-PLA film was confirmed by ATR-FTIR analysis. Furthermore, smoother surface morphology of CA-PLA film than that of pure PLA film were observed through field emission scanning electron microscopy (FESEM). There were no significant (p > 0.05) changes in WVP and OP of CA-PLA film by addition of CA.

Significance: Developed CA-PLA film performed antibiofilm and antimicrobial activity against *S*. Typhimurium and *S*. Enteritidis contamination on chicken breast and beef without any adverse effect on their qualities, demonstrating that it can be used as promising active packaging to prevent *Salmonella* contamination in meat industry.

P2-35 Extended Spectrum Beta-Lactamase (ESBL)-producing *Salmonella enterica* in Agricultural Water of the Metropolitan Region of Chile

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❖ ***Developing Scientist Entrant***

Introduction: The presence of *Salmonella* strains that produce Extended-Spectrum Beta-Lactamases (ESBLs) poses a significant risk to public health. These strains exhibit resistance to antimicrobials that are typically relied upon to treat severe infections.

Purpose: This study aims to evaluate the presence and distribution of ESBL-producing *Salmonella enterica* in surface waters of the Metropolitan Region of Chile.

Methods: We conducted an analysis on 501 isolates of *Salmonella enterica* obtained from 60 locations along the Maipo and Mapocho rivers in central Chile. These isolates were collected from April-2019 to July-2021. Serovars were identified using whole genome sequencing. Furthermore, we assessed the minimum inhibitory concentrations (MIC) using 14 antibiotics, including Aminoglycosides, β-lactams, β-lactamase inhibitors, Fluoroquinolones, Phosphonates, and Diaminopyrimidine/Sulfonamide. The determination of MIC and ESBL production was conducted using the agar dilution technique in accordance with CLSI guidelines.

Results: Out of the 501 isolates analyzed, 11,4% (57/501) exhibited a multidrug-resistant (MDR) phenotype and 9.4% (47/501) had an ESBL-producing phenotype. Notably, all of these were serovar Infantis. 18% (88/501) of the total isolates recovered were identified as *S.* Infantis, with 57% (50/88) exhibiting a MDR profile, with a higher presence in the Maipo, 63.4% (26/41), compared to the Mapocho, 51.1% (24/47). The presence of ESBL-producing *Salmonella* Infantis reached 63.4% (26/41) in the Maipo and 45% (21/47) in the Mapocho. The presence of ESBL-producing *S.* Infantis in the rivers of the Metropolitan Region of Chile represented 53.4% (47/88) of the total isolates of *S.* Infantis, equivalent to 9.4% of the total isolates analyzed.

Significance: This study underscores the extensive prevalence of ESBL-producing *Salmonella enterica*, specifically serovar Infantis, within agricultural water across all seasons in the Metropolitan Region of Chile. These results emphasize the pressing necessity to delve into dissemination patterns, prompting the creation of robust surveillance strategies and targeted control measures to ensure the safety of our food supply.

P2-36 Treatment of *Listeria monocytogenes and Salmonella enterica* Biofilms with Antimicrobial Blue Light

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Introduction: Contamination with *L. monocytogenes* and *Salmonella* is a serious public health risk. Current plant sanitation programs are often not capable of controlling pathogen biofilms on equipment surfaces. A possible intervention to reduce the biofilm viability is the use of antimicrobial blue light (aBL).

Objective*:* Determine optimal aBL doses needed to inactivate biofilms of *Listeria monocytogenes* and *Salmonella* on stainless steel, glass, and high-density polyethylene (HDPE) surfaces.

Methods: Five-strain mixtures of *L. monocytogenes* and *Salmonella* were used independently. Strains were grown in tryptic soy agar at 37 °C and mixed in tryptic soy broth (TSB). Biofilms were grown in a Drip Flow Biofilm Reactor at room temperature. Mixed TSB cultures were incubated in reactor cells with coupons for 24 h before starting TSB flow. Coupons were removed after 48 h, and wet biofilms were exposed to aBL (405 nm) treatment. Also, coupons were dried and exposed to aBL. Coupons were sonicated in buffer and counts were determined by plating on TSA. Log CFU/coupon were calculated and averaged for replicates from at least two independent experiments. Statistical analyses were performed using Student t-Test.

Results: The treatment with 4,320 J/cm2 of wet *Listeria* and *Salmonella* biofilms reduced the viable count > 4.0 Log CFU on all three surfaces, but dry biofilms exposure only reduced viability by < 2.0 log CFU (p < 0.05). The minimum dose to reduce the viable count by > 2.0 Log for wet *L. monocytogenes* was 1,080 J/cm2 on each surface, and for *Salmonella* on stainless steel and glass. However, only half of the dose was needed to reduce the same level of *Salmonella* biofilms on HDPE.

Significance: This study indicates that aBL inactivated *Listeria* and *Salmonella* biofilms on different surfaces and this could be a novel technology for the improvement of microbial safety in food processing plants.

P2-37 Cross-Resistance to 14-,15- and 16 Membered Ring Macrolides in *Salmonella* and *Campylobacter*

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Introduction: Macrolides are currently ranked as critically important antimicrobials by both the World Health Organization and the U.S. Food and Drug Administration. Although mechanisms of resistance to macrolides are extensively studied, their relationship to various macrolide ring structures is not well defined.

Purpose: This project aimed to gain a better understanding of how resistance determinants in *Salmonella* and *Campylobacter* contribute to resistance phenotypes to 14-, 15- and 16-membered ring macrolides.

Methods: A total of 126 azithromycin-resistant (Azi^R) and -susceptible (Azi^S) *Salmonella* (*n* = 45) and *Campylobacter* (*n* = 81) isolates from food animals at slaughter and processing, and retail meat samples were selected for antimicrobial susceptibility testing using a custom panel and whole genome sequencing.

Results: Seven functional macrolide resistance determinants, including *erm* (42), *mef*(C), *mph*(A), *mph*(E), *mph*(G), *msr*(E), and one point mutation (*acrB*_R717L) were identified in Azi^R *Salmonella*, among which *mph*(A) was the most prevalent (61.3%). These determinants resulted in up to 256-fold minimum inhibitory concentration (MIC) increases against 15-membered ring azithromycin and gamithromycin, and up to 32- and 16-fold MIC increases for 14-membered ring erythromycin. *Salmonella* with *erm* (42) or *acrB*_R717L showed up to 128-fold MIC increase for 16-membered ring macrolide tildipirosin, as compared to isolates which were susceptible or carrying other macrolide resistance genes. Compared to control isolates, all Azi^R *Campylobacter* had higher MICs (128 to 4,096-fold) than the various membered ring macrolides.

Significance: Our study revealed that the wide spectrum of macrolide resistance in Salmonella is independent of macrolide ring structure, with the exception of erm(42) and acrB_R717L, whereas macrolide resistance in Campylobacter is primarily due to a 23S rRNA mutation. The findings from our study will help guide national and international efforts aimed at preserving the effectiveness of macrolides, a critically important class of antimicrobials used both in human and animal health.

P2-38 Antimicrobial Resistance Genes in *Salmonella enterica* Obtained from Surface Waters of Two Food-Production Regions in the State of Rio De Janeiro, Brazil

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Introduction: In livestock-producing regions, surface waters (SuWa) may transport microorganisms carrying antimicrobial resistance (AMR) genes.

Purpose: To investigate acquired AMR determinants in *Salmonella enterica* obtained from SuWa from two Rio de Janeiro State food-producing regions.

Methods: We sampled two geographical areas of the Rio de Janeiro State from November 2019 to March 2022 on six occasions in each region. Region 1 (R1) produces chicken and vegetables, and Region 2 (R2) focuses on dairy. SuWa samples were filtered *in situ* using the modified Moore Swab technique. *Salmonella* isolation and identification followed FDA/BAM procedures. *Salmonella* isolates were sequenced at CFSAN/FDA on Illumina NextSeq, and genomic information was submitted to NCBI. Genomes were characterized for serovar prediction with SISTR, and we identified the presence of AMR genes with NCBI/AMRFinderPlus.

Results*:* 121 non-clonal isolates were obtained from 69 SuWa samples in 49 sites of R1. In R2, 96 isolates were recovered from 65 SuWa samples in 48 sites. A total of 42 isolates (19%), 18 different serovars, carried acquired resistance genes [R1 (20), R2 (22)]. The most frequently identified genes were associated with resistance to fosfomycin [*fosA* (21)]; tetracycline [*tet(A)* (11), *tet(B)* (3) and *tet(C)* (1)]; quinolones [*qnrB* (13) and *qnrC* (1)] and aminoglycosides [*aad* (5) and *aph* (9)]. Moreover, we identified genes conferring resistance to beta-lactams [*blaCMY* (5) and *blaTEM* (4)]; sulfonamide [*sul1* (1) and *sul2* (7)]; trimethoprim [*drfA* (5)] and florfenicol (*floR* (4)]. Fifteen isolates (7%) were multidrug-resistant, with AMR genes for three or more antibiotic classes; twelve of them were collected in R1. These strains from R1 belong to eight different serovars and were isolated from nine sites and four samplings.

Significance: Multidrug-resistant *Salmonella* is uncommon in SuWa of Rio de Janeiro state but occurs in different serovars. These strains were more prevalent in R1, which intensively produces chicken and vegetables.

P2-39 A Targeted Phage Cocktail Designed for *Salmonella* Infantis Effectively Reduced the Presence of This Emerging Pathogen in Chicken Breast without Compromising the Quality of the Meat

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❖ ***Developing Scientist Entrant***

Introduction: The rise of *Salmonella* Infantis poses a global concern, particularly within the poultry industry. In certain regions, *S.* Infantis stands out as a prevalent and extensively distributed serovar, exhibiting multidrug resistance. Addressing this issue calls for innovative interventions.

Purpose: The objective of this study was to validate the efficacy of a phage cocktail (PC) in mitigating MDR *S.* Infantis in chicken breast while evaluating its influence on meat quality.

Methods: A PC of three phages specific to *S.* Infantis, underwent validation through testing effectivity on 71 *S.* Infantis strains isolated from retail poultry meat. The stability of the PC was assessed *in vitro* at 0ºC in a marinate solution and peracetic acid (PAA) (200 ppm) solution The application model included two approaches with titer of 10^8PFU/mL: injection and spraying the PC. Both models were tested in 25g of chicken breast inoculated with 10^4 CFU of SI. Quantification through Most Probable Number (MPN) and the evaluation of quality parameters (pH, color, and aerobic counts) were performed over 4 days at 0ºC. Experiments were conducted in triplicate and the statical analysis by GraphPadv10.2.1.

Results: The PC showed efficacy against 98% of tested SI strains. The phage activity remained stable at 0ºC and in the marinate solution, but a significant decrease occurred with 200 ppm PAA within 1 hour. Injection showed a significant phage reduction in comparison with the spray model. For the infection model, spray was used. *Salmonella* detection was below the limit (<3 MPN per g) on days 0 and 4. Throughout the 4-day period, chicken breast pH remained constant at 6 in both control and phage-treated groups, with no observable color differences. The cocktail-treated group exhibited a significant reduction in total aerobic count compared to the control on day 4.

Significance: The developed phage cocktail demonstrated promise in effectively controlling MDR *S.* Infantis in chicken breast under standard storage conditions while maintaining meat quality.

P2-40 Determination of the Concentrations of Salt, Lactate, and Diacetate That Inhibit the Growth of *Salmonella* in Meat Products

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Introduction: Refrigerated cooked or shelf-stable dried meat products may be contaminated with *Salmonella* in the post-lethality processing environment, and subsequent improper handling may allow the pathogen to grow and cause foodborne illnesses.
Purpose: This study was conducted to determine the concentrations of salt, sodium lactate, and sodium diacetate that may inhibit the growth of *Salmonella* in meat products.
Methods: Sterilized tryptic soy agar (TSA, 200 µl) formulated with a combination of salt (3-8%, a_w 0.98-0.93), lactate (0-2.4%), and diacetate (0-0.25%) and inoculated with a *Salmonella* cocktail was placed into 96-well microplates and incubated at 37°C for 7 days. After incubation, a well with or absent of *Salmonella* colonies was denoted as growth or no-growth. The effects of salt, lactate, diacetate, and their interactions on the growth responses were analyzed by logistic regression. The formulations that had complete no-growth response in microplates were tested in cooked and *Salmonella*-inoculated ground beef for applications in meat products.
Results: Logistic regression analysis showed that salt, lactate, and diacetate and their interactions significantly (p<0.05) affected the growth probability of *Salmonella* in TSA. The minimal growth-inhibiting concentrations were 3% salt (a_w 0.98) with 0.8% lactate+0.2% diacetate or 1.6% lactate+0.1% diacetate, 4% salt (a_w 0.97) with 2.4% lactate, 5% salt (a_w 0.96) with 0.25% diacetate, 6% salt (a_w 0.95) with 0.8% lactate+0.15% diacetate or 0.2% diacetate, and 7% salt (a_w 0.94) with 0.8% lactate or 0.15% diacetate. Salt alone at 8% (a_w 0.93) inhibited the growth. These growth-inhibiting formulations were also shown to inhibit the growth of *Salmonella* in the meat samples.
Significance: This study identified the effects and growth-inhibiting concentrations of salt (or equivalent aw), lactate, and diacetate on *Salmonella* in TSA and cooked meat. The information may be used for formulating refrigerated and shelf-stable meat products to reduce *Salmonella* risk.

P2-41 Evaluating the Inhibitory Impact of a Novel Microwave Interventions on Pre-Packed Flour Tortillas Inoculated with Salmonella and Mold Spore Strains

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Introduction: Food security initiatives require the availability of alternative interventions validated for mitigating pathogens and spoilage strains. Corn tortillas render traditional interventions impractical at times because of detrimental quality effects. Investigating alternatives like microwaves emerges as an avenue to safeguard the safety of this food product.
Objective: Evaluate the potential effectiveness of MicroZap (microwave technology) in controlling the growth of *Salmonella* and mold strains shelf-life effects of flour tortillas.
Methods: Flour tortilla samples were inoculated with 250 µL of a *Salmonella* cocktail containing three strains (*S. Senftenberg, S. Typhimurium, and S. Newport*) and a mold cocktail containing two spoilage strains (*Penicillium crustosum* and *Aspergillus niger*). In total, 20 packages were inoculated for each target microorganism, with 5 packages per treatment level or the control group. The treatment levels were as follows: Tr 1 = 305.8 kWs at 3.5 cm/s belt speed, Tr 2 = 340.2 kWs at 3.1 cm/s belt speed, Tr 3 = 369.8 kWs at 2.9 cm/s belt speed.
Results: The *Salmonella* concentration in the inoculated control samples measured 5.26±0.12 log10CFU/g. Reductions, ranging from 0.93 to over 2.82, were achieved with incremental power levels in the treatments. For Tr 3, two *Salmonella* inoculated samples received 4.5% higher energy (386.5 kWs), which could explain the high variability in resulting *Salmonella* counts. The average mold concentration in the inoculated control samples was 4.93 log CFU/g, exceeding the calculated inoculum level. Given that untreated samples exhibited visible spoilage the day after production, it is possible that the samples were already contaminated at an estimated level of 1 log CFU/g. Treatments led to mold reductions ranging from 1.91 to over 3.33, particularly at the highest microwave power level.
Significance: Evaluating a novel microwave intervention technology in artisanal products can affect bacterial survival of pathogens like *Salmonella* and the persistence of spoilage mold spores. More studies are necessary to determine the feasibility of microwave intervention on tortilla products.

P2-42 Gallic Acid Inhibits the Growth of Antibiotic-Resistant *Salmonella*

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Introduction: *Salmonella enterica* has increasingly exhibited resistance to various antibiotics (AMR) traditionally used for treatment of foodborne infections and may lead to cross resistance to food grade disinfectants and persistence of *Salmonella* isolates in the food processing environment. This situation underscores the need for identification of new food grade antimicrobials to control the presence of AMR *Salmonella*.
Purpose: To assess the efficacy of phenolic acids as a natural food grade antimicrobial to inhibit the growth of AMR *Salmonella*.
Methods: Animal and environmental *Salmonella* isolates belonging to serotypes Hartford, Poona, Alachua, Muenchen, and Uganda were subjected to short-read paired-end whole genome sequencing (WGS) on the Illumina MiSeq platform, followed by analysis of antimicrobial resistance genes using the Comprehensive Antibiotic Resistance Database (CARD). Each *Salmonella* isolate was tested in pure culture to determine the minimum inhibitory concentration (MIC) against gallic acid. A stock solution of gallic acid was diluted in tryptic soy broth (TSB) to final concentrations of 16, 12, 8, 6, 4, and 2 mg/ml and added to individual wells of a 96 well microtiter plate. Two hundred microliters of fresh cultures of each *Salmonella* isolate was added to individual wells of the microtiter plate, which was placed into an incubating spectrophotometer for 20 hr at 37°C with agitation. Absorbance readings were obtained every 15 minutes at OD600nm.
Results: WGS and bioinformatic analysis with CARD identified the presence of aminoglycosides resistance (aac(6')-Iaa, aac(6')-Iy), fosfomycin resistance (fosA7) and multidrug resistance efflux pump associated genes (mdtK, mdsA, mdsB, mdsC, gloS, sdiA) in the *Salmonella* isolates. MIC assays indicated that all *Salmonella* isolates were susceptible to 8 mg/ml of gallic acid.
Significance: These results highlight the potential utility of phenolic (gallic) acid as an antimicrobial to inhibit AMR *Salmonella*. Future studies will develop practical applications for use during fresh produce processing activities.

P2-43 Effectiveness of Bacteriophage against Antibiotic Resistant (MDR) *Salmonella* Infantis from Poultry

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Introduction: Lately, *Salmonella* Infantis and its multi-drug resistant (MDR) variants has become one of the predominant *Salmonella* serotypes in the US poultry sector. Bacteriophage has emerged as a promising alternative pathogen control measure in the poultry industry with its application at various pre- and post-harvest stages.
Purpose: To evaluate the effect of a cocktail bacteriophage against MDR *S.* Infantis.
Methods: Two *S.* Infantis strains (~5 log in 2X TSB) resistant to Tetracycline, Nitrofurantoin, tobramycin, and Gentamycin were subjected to varying concentrations [1, 10, 100, 500, 1000, 2000, 3000, 4000, 5000, 6000, and 7000, 8000, 9000, 10,000 MOI and control] of Salmofresh®, a cocktail of 6 monophage in a 96-well plate at 37°C and room temperature (25°C). The OD_{600} (EPOC-2 BioTek®) values taken over 22h at 30-minute intervals, were plotted against

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time and analyzed for the lag phase. Duplicate (3 wells per replicate) experiments were performed and the lag intervals were calculated using the means. The extension of the lag phase was recorded based on findings that bacteria enter the log phase upon OD_{600} exceeding 0.2.

Results: At all tested levels of bacteriophage, both *S*. Infantis strains exhibited significant ($p \leq 0.05$) growth inhibition at 37 °C and 25 °C. However, at 37 °C, *Salmonella* entered an exponential phase (OD600 > 0.2) at ~9h briefly before dropping again at ~11h. This phenomenon was more prominent with the exponential phase continuing past 21h even at higher MOI (10,000) with Gentamycin resistant strain compared to the susceptible strain. A similar momentary surge in the *Salmonella* population was observed at 25 °C but after ~2h of incubation.

Significance: The bacteriophage-based intervention seemed to be effective in controlling MDR *Salmonella*. However, the re-growth after initial inhibition by the bacteriophage in these MDR strains needs to be further analyzed.

P2-44 Effect of Sublethal Exposure of Peroxyacetic Acid (PAA) against Multi-drug Resistant *Salmonella* Infantis

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❖ ***Developing Scientist Entrant***

Introduction: Multi-drug resistant (MDR) *Salmonella* Infantis has emerged as one of the major *Salmonella* serotypes in the US poultry industry. The widespread use of Peroxyacetic acid (PAA) in poultry processing necessitates a comprehensive evaluation of its impact on MDR S. Infantis to gauge potential resistance.

Purpose: To study the effect of sublethal PAA on MDR *S*. Infantis at different temperatures.

Methods: Two poultry-origin MDR *S*. Infantis strains (~5 log in 2X TSB) were subjected to varying PAA concentrations [0 ppm (control),10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 ppm] in a 96-well plate. The OD_{600} readings at 37°C and room temperature (25°C) were recorded (EPOC-2 BioTek®) over 22h at 30-minute intervals, and the values were plotted against time and analyzed for the lag phase. The experiment was conducted in duplicate (4 wells per replicate) and the means were used to calculate the lag interval. The duration of the lag phase was measured with the assumption that bacterial cells enter the log phase when the OD_{600} exceeds 0.2.

Results: Both *S*. Infantis strains exhibited increasing growth inhibition with rising PAA concentrations both at 37 °C and 25 °C. Notably, at 37°C the control sample had a lag phase of approximately 6.5 hours, while at 50, 60, 70, 80, and 90 ppm it extended to *ca.* 10, 10.5, 13, 17, and 22 hours respectively. At 100 ppm, PAA induced growth inhibition until 22h. However, at 25 °C, the control lag phase extended to around 12 hours, with PAA concentrations above 40 ppm inhibiting bacterial growth until the study period.

Significance: The revival of PAA-stressed MDR *Salmonella* after an extended period could lead to false negatives in conventional detection methods. A further study, on the possible linkage between antibiotic resistance and biocide resistance, is warranted.

P2-45 Peroxyacetic Acid Reduces *Salmonella* Load on the Surface of In-Shell Pecans and Prevents Cross-Contamination during Conditioning

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Introduction: In-shell pecans are generally conditioned in water prior to shelling which presents the potential for foodborne pathogen cross-contamination. The use of sanitizers in conditioning water can be an effective way to prevent cross-contamination.

Purpose: The objective of this study was to determine the effectiveness of peroxyacetic acid (PAA) in conditioning water at reducing *Salmonella* loads and preventing cross contamination on in-shell pecans.

Methods: In-shell pecans (200 g) were inoculated with 40 ml of a rifampicin resistant 5-strain *Salmonella* cocktail. Inoculated pecans (~7 log CFU/g) were placed, with uninoculated pecans to determine cross-contamination, in water treatments containing varying concentrations of PAA (0, 30, and 90 ppm) and were sampled 0, 2, 15, and 60 minutes following treatment. Pecans were diluted with buffered peptone water, hand massaged, and plated onto tryptic soy agar containing rifampicin. Data (n=6) was analyzed using ANOVA and Tukey's HSD ($p \leq 0.05$).

Results: There were significant ($p<0.05$) *Salmonella* reductions at each timepoint on pecans conditioned in water containing 30 and 90 ppm PAA with the final reduction being 3.4±0.9 and 4.2±0.5 log CFU/g respectively after 60 minutes. At 2 minutes, there was a significant difference in *Salmonella* reduction on pecans treated with 30 and 90 ppm PAA (1.8±0.7 and 2.8±1.0 log CFU/g respectively), though there were no significant differences between the *Salmonella* reductions at the 15- and 60-minute timepoints. There was an observed cross-contamination of 4 logs onto the uninoculated pecans in 0 ppm PAA after 60 minutes, while the *Salmonella* levels were below the limit of detection (<0.3 log CFU/g) on uninoculated pecans at 30 and 90 ppm PAA.

Significance: PAA is an effective sanitizer at preventing cross-contamination and also reducing *Salmonella* populations on in-shell pecans during conditioning. PAA concentrations between 30 and 90 ppm only had a significant influence on *Salmonella* reduction in the first 2 minutes of treatment.

P2-46 Comparative Evaluation of the Efficacy of Organic Sanitizers against *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli* O157:H7 and Leafy Green Native Microbiota on Different Food Contact Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: The effectiveness of sanitizers in the food industry is influenced by several factors, including the surface of application. Overuse of chlorine-based chemical sanitizers can result in the development of resistance among the microbes. These chemical sanitizers can have adverse effects on human health, while also being corrosive to equipment and other surfaces.

Purpose: The efficacy of organic and plant-based sanitizers on food contact surfaces against foodborne pathogens and leafy green native microbiota was investigated.

Methods: Coupons of stainless steel 304, high density polyethylene (HDPE), polyvinyl chloride (PVC) and polycarbonate (PC) were inoculated with pathogens (*Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli*O157:H7) and leafy green native microbiota (from spinach, cut romaine and arugula). The inoculated coupons were dried for 30 minutes and then treated for two minutes with test sanitizers including a 0.5% essential oil microemulsion, 5% plant extract solution and 20% commercial organic sanitizer. Coupons were dipped in neutralizing broth, vortexed and aliquots were plated on tryptic soy agar for enumeration.

Results: The essential oil microemulsion and commercial organic sanitizer were very effective in reducing microbial populations below the detection limit (<1 log CFU/coupon). Plant extract showed variable efficacy based on the microbe and type of coupon. For *L. monocytogenes*, the plant extract resulted in reduction below detection limits and for *Salmonella*, reductions ranging from 1.3 to 2.5 log CFU/coupon were observed. For *E. coli* O157:H7, reductions were below detection limits for Stainless Steel 304 and PC, and 3.5 and 4.1log CFU/coupon for HDPE and PVC, respectively. For all the native leafy green microbiota, reductions ranged from 3.6 to 5.1 log CFU/coupon for different food contact surfaces, while spinach microbiota were below detection levels on PVC and PC.

Significance: Results indicate that organic sanitizers can potentially be used to sanitize food contact surfaces to reduce cross-contamination in food processing plants.

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P2-47 Antibacterial Activity and Mechanism of *Citrus aurantium* Extract against *Salmonella* Typhimurium

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Introduction: Citrus aurantium extract (CAE) has antibacterial activity against various foodborne pathogen, such as *Salmonella* Typhimurium, so it is used as a natural antibacterial agent.

Purpose: This study was performed to investigate the antibacterial activity and mechanism of CAE against *Salmonella* Typhimurium.

Methods: A cocktail of three strains of *S*. Typhimurium (ATCC 19585, 48971, 14028) was prepared to achieve a final concentration of 1.07×10^8 CFU/ml. The minimum inhibitory concentration (MIC) for *S*. Typhimurium was determined using the broth dilution method. Fluorescent dyes, namely Propidium iodide (PI), Bis-(1,3-dibutylbarbituric acid) trimethine oxonol ($DiBAC_4(3)$), and 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA), were employed to measure membrane damage, membrane potential changes, and intracellular ROS, respectively. Bacterial suspension (500 µl) was treated with 500 µL of CAE (1/4 MIC, 1/2 MIC, MIC, 2 MIC) for 15 min at 37°C, while the control group was treated with 0.85% saline. After adding a fluorescent dye and causing a dark reaction, the fluorescence value of the supernatant (200 µl) was measured after centrifugation at 8000 ×g for 5 min at 4°C.

***Results*:** The control exhibited lower relative fluorescence units (RFU) values for membrane damage (1.53 ± 0.10 RFU), membrane potential (7.66 ± 0.09 RFU), and intracellular ROS (0.24 ± 0.01 RFU), compared to cells treated with 2 MIC, which showed higher values for membrane damage (36.78 ± 0.06 RFU), membrane potential (366.47 ± 0.52 RFU), and intracellular ROS (3.36 ± 0.04 RFU). In all experiments, RFU values increased with higher concentrations of CAE treatment. Therefore, CAE induces cell death by damaging the cell membrane and increasing both membrane potential and intracellular ROS levels.

Significance: CAE demonstrated effective control over *S*. Typhimurium, suggesting its potential use as a natural antibacterial agent in various food applications.

P2-48 Antimicrobial Efficacy of Far UV-C (222 nm) against *L. monocytogenes* and *Salmonella enterica* Contaminated HDPE Surfaces

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Introduction: *L. monocytogenes and S. enterica* can attach to food contact surfaces and cross contaminate food products. Far UV-C (222 nm) treatment is effective against foodborne pathogens, while being harmless to mammalian cells due to limited penetration depth.

Purpose: This study investigates the efficiency of far UV-C (222 nm) in reducing *L. monocytogenes* and *S. enterica* population on high-density polyethylene (HDPE) surfaces

Methods: Sterile HDPE coupons (8.6×5.3 cm) were inoculated with 9 log CFU/cm^2, of a 5-strain cocktail of *L. monocytogenes* and *S. enterica.* Coupons were exposed to far UV-C (222 nm) at a light intensity of 381.6 mJ/cm^2, 1526.4 mJ/cm^2, and 3052.8 mJ/cm^2 for 1, 4, and 8 h. On completion of the treatment, coupons were sonicated for 5 min, and enumerated on tryptic soy agar with 0.6% yeast extract (TSAYE) and modified oxford agar (MOX) plates after incubation at 37°C for 48 h. Inoculated coupons kept at dark and exposed to a white, fluorescent bulb light (0.2 J/cm^2; FBL) were considered as positive and negative controls. Experiments were conducted as three biological and three technical replicates. Statistical significances were determined using ANOVA.

Results: Significant reduction in bacterial population on HDPE coupons exposed to Far UV-C (222 nm) ($p \leq 0.05$) was observed compared to both FBL and dark treatment conditions. An exposure period of 1,4, and 8 h led to a significant reduction of *L. monocytogenes* population by 2.49±0.33, 2.44±0.09 and 3.26±0.33 log CFU/cm^2, while *S. enterica* on HDPE coupons exhibited a reduction of only 1.18±0.17, 0.71±0.12 and 1.01±0.32 log CFU/cm^2 ($p \leq 0.05$).

Significance: Treatment time is an important factor contributing to the efficacy of far UV-C (222 nm) treatment and the susceptibility to far UV-C (222 nm) is bacterial-specific.

P2-49 Isolation, Identification, and Characterization of *Bacillus* Strains That Could Prove Useful for Both Probiotic Health Benefits and Food Safety Applications

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❖ ***Developing Scientist Entrant***

Introduction: Spore-forming *Bacillus* spp. have been extensively used as probiotic supplements due to their health benefits.

Objective: Objective of this study is to evaluate antibacterial potential of *Bacillus* strains isolated from retail and commercial probiotic samples against foodborne pathogens and to determine the antimicrobial nature of the activity.

Method: *Bacillus* spp. were isolated from commercial probiotic products and retail foods and identified by RT-PCR amplification of 16S rRNA using universal 7F/928R and 515F/1541R primers. Antibacterial activity was evaluated against *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, and Clostridium perfringens* using patch and overlay methods. Spore crops from *Bacillus subtilis DE111, Bacillus coagulans* MTCC *5856, Bacillus coagulans GBI-30/6086* were harvested to perform thermal death kinetics and food processing applications.

Results: DNA sequencing and 16S sequence analysis confirmed 2 isolates from hot sauce as *B. subtilis* and *B. licheniformis* and three strains from commercial probiotics as *B. coagulans* (GBI-30/6086), *B. coagulans* (MTCC 5856), and *B. subtilis* (DE111) demonstrated statistical validity of identification based on >98% query and >99% identity match to 1,400 bp of 16S rRNA sequence. All *Bacillus* strains exhibited elevated antibacterial activity against *Clostridium perfringens* ATCC 12919, 8237, and 13124, followed by *Listeria monocytogenes* PMM 99-38 and JAG-167. None were inhibitory towards *B. cereus* or *S. aureus*. Spore crops were evaluated for enumeration, with and without heating (80 °C, 15 min), to distinguish total CFU/ml from spore-related counts for spore recovery of up to 9.12 log CFU/ml (*B. subtilis* DE111), 9.6 log CFU/ml (*B. coagulans* GVI-30/6086, and 10.25 log CFU/ml (*B. coagulans* MTCC 5856).

Significance: Most work done with bacterial antimicrobials has focused on bacteriocins from LAB. Antimicrobials from probiotic *Bacillus* spp. may simultaneously serve as both food biopreservatives and provide probiotic dietary health benefits when supplied in foods.

P2-50 Sodium Lactate Prevents Growth of Proteolytic and Non-Proteolytic *Clostridium botulinum* in Uncured Turkey Products More Effectively Than Sodium Diacetate or Propionate

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Introduction: Sodium nitrite is an effective inhibitor of *C. botulinum* growth and toxin production in meats; however, manufacturers seek clean-label alternatives. Sodium lactate, diacetate, and propionate are examples of antimicrobial components within clean-label fermentates potentially useful at inhibiting *C. botulinum* toxin production in ready-to-eat meats.

Purpose: To examine the efficacy of lactate, diacetate, and propionate on inhibition of *C. botulinum* growth and toxin production in uncured turkey.

Methods: Ground turkey (72-74% moisture, pH 6.20, 1% salt) formulated without antimicrobials (positive control), with 150 ppm sodium nitrite and 250 ppm erythorbate (negative control), sodium lactate (1.5%; 3%), sodium diacetate (0.25%; 0.5%) and sodium propionate (0.25%; 0.5%) was inoculated with 2.5-3.0 log spores/g of a cocktail containing proteolytic and non-proteolytic *C. botulinum* spores. Samples (50 g) were vacuum-packaged, cooked to 71°C, chilled, and incubated at 20°C for 14 days. *C. botulinum* were enumerated daily on Differential Reinforced Clostridial Agar (DRCA). Botulinum toxin was detected using DIG-ELISA and mouse bioassay.

Results: The earliest time to toxicity observed in the control formulation (without antimicrobials) was on day 2 (0.99 log increase). Although 1.5% sodium lactate supported toxin at day 4 (2.26 log increase), no toxin was observed with the 3% treatment or with the negative control throughout 14 days storage

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at 20°C. Sodium diacetate treatments were toxic on days 2 (1.47 log) and 3 (1.48 log) for 0.25% and 0.5% treatments, respectively. Similarly, toxin was detected in sodium propionate treatments on day 2 (3.64 log) and day 3 (1.48 log) for 0.25% and 0.5%, respectively.

Significance: This study highlighted that when used at levels acceptable in processed meats, sodium lactate exhibits greater inhibitory effects of *C. botulinum* growth and toxigenesis in uncured turkey products compared with sodium diacetate and propionate. These data support the development of effective clean label antimicrobial fermentates targeting *C. botulinum*.

P2-51 Evaluation of the Activity of a Coffee Pulp Extract on the Germination of *Bacillus cereus* Spores

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Introduction: The processing of coffee fruit to obtain the bean from which the beverage is made generates a series of by-products that contain bioactive compounds of interest. The effect of these compounds on bacterial spores has not been extensively studied.

Purpose: To evaluate the activity of a coffee pulp extract on the germination of *B. cereus* spores.

Methods: Three samples of coffee pulp collected in Tres Ríos; Costa Rica were used. The methodology described by Mingo et al., 2016, (slightly modified), was used to obtain the extracts. One loop of a *Bacillus cereus* (strain ATCC14579) suspension was streaked onto Nutritive Agar plates and incubated at 30°C for 7 days under aerobic conditions. After incubation, all bacterial growth (spores) was collected using a Digralski loop and resuspended with 0.85% NaCl. The bacterial spore suspension was placed into sterile test tubes and heated at 75°C for 20 min in a water bath to eliminate vegetative cells. To evaluate the germination, 100 ml of TSB, 50 ml of spores and 50 ml of treated spores (75°C for 20 min) or vegetative cells was mixed using a 96-well microplate. The samples were homogenized and incubated at 30°C for 6 hours. Then, 50 ml of coffee pulp extract was added and incubated again at 30°C for 48 hours. After this time, *Bacillus cereus* spores were counted and converted to log CFU/mL.

Results: Coffee pulp extract inhibited the germination of *B. cereus* spores when the bacterial population densities were between 100 and 10,000 spores/ml and the concentration of the extract was 1 mg of phenolic compounds/ml.

Significance: This extract could be used as a natural antimicrobial that can contribute to the control of *B. cereus* populations in food products and production environments.

P2-52 Genomic Profiling of Antimicrobial Resistance in Retail Meat Isolates from Kosovo

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Introduction: The global escalation of antimicrobial resistance in foodborne pathogens is a significant concern. This study, centered on retail meat isolates from Kosovo, a region where research in this realm is largely lacking, employs the NARMS protocol for systematic isolation and characterization prior to genomic characterization.

Purpose: The primary objective is to characterize the genomic composition and antimicrobial resistance profiles of retail meat isolates, offering valuable insights into the prevalence and distribution of resistance genes within the food chain.

Methods: Retail meat isolates, including *Escherichia coli* and *Acinetobacter pittii*, were obtained following the NARMS protocol designed for sample acquisition. Genomic analysis utilized whole genome sequencing short read data. Quality trimming with Trimmomatic, de novo assembly using SPAdes, and annotation with Prokka were sequentially applied for genomic reconstruction and identification of genes and features.

Results: *Escherichia coli* isolates exhibited diverse plasmid content, including IncFIA, IncFIB, IncQ1, and p0111. Antimicrobial resistance genes like $bla_{CTX\text{-}M\text{-}55}$, *dfrA36*, *sul2*, and *aph(3")-Ib* were identified. *Acinetobacter pittii* isolates displayed resistance genes such as $bla_{OXA\text{-}421}$, $bla_{ADC\text{-}151}$, and $bla_{OXA\text{-}826}$. *Enterobacter cloacae* isolate harbored resistance genes *oqxB9*, *oqxA9*, and $bla_{ACT\text{-}12}$.

Significance: By employing standard isolation protocols for selected AMR bacteria and conducting extensive genomic characterization, this study contributes to a thorough genomic understanding of retail meat isolates from Kosovo. The revealed diverse plasmid profiles and antimicrobial resistance patterns enhance our insight into resistance dissemination in the local food supply chain, facilitating the formulation of effective strategies to combat the global challenge of antimicrobial resistance.

P2-53 Use of Carvacrol as Marination Additive for Post-Production Control of Foodborne Pathogens in Lamb

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❖ ***Developing Scientist Entrant***

Introduction: Lamb meat production in the US alone was over 104 million pounds in 2023; however, there is still a lack of literature on post-production microbial controls for lamb meat.

Purpose: In this experiment, the potential of using carvacrol as a marinade add-in was explored as an avenue to control the growth of both *E. coli O157:H7* and *Salmonella* species in lamb meat.

Methods: Commercial marinade mix was prepared according to package directions, then divided into three groups: no carvacrol, 0.5% (v/v) carvacrol added, and 1.0% (v/v) carvacrol added. Lamb meat was cut into approximately 10g pieces and submerged into their respective marinade treatments for 1 hour or left unmarinated as negative controls. Samples were inoculated with a cocktail of three *E. coli O157:H7* strains and five *Salmonella* species. Samples were refrigerated and bacterial growth was quantified at days 0, 3, 5 and 7 post-inoculation using culture-based methods. Additionally, non-inoculated samples were used to determine L*a*b* color metrics on days 5, 7 and 12, and pH on days 0, 3, 5, 7, and 11. All experiments were performed in triplicate.

Results: Results showed that there was no significant ($p>0.05$) log reduction for either *E. coli O157:H7* or *Salmonella* in any treatment group. However, all marinades prevented growth ($p<0.05$) of the bacteria compared to the negative control. While the negative control samples pH rose throughout the experiment, all marinade groups maintained a pH between 5.2-5.4; this lower pH may have contributed to these samples limited bacterial growth. The yellowness (b*) of non-marinated meat was also much lower than marinaded meat.

Significance: The results demonstrate that though carvacrol does not provide additional anti-bacterial properties to marinades, marinades can still be used to prevent growth of both E. coli and Salmonella in lamb meat and could be used with other hurdle technology to increase post-production food safety.

P2-54 Investigating the Antimicrobial Activity of Turmeric and Rosemary Essential Oils against Predominant Microorganisms Isolated from Raw Beef Obtained from Markets in Accra, Ghana

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Introduction: The presence of foodborne pathogens such as *Salmonella* and *Escherichia coli* in raw beef is a major public health concern.
Purpose: This study investigated the prevalence of Salmonella and Escherichia coli in raw beef sold in open markets and malls in Accra. The effectiveness of antimicrobial agents (rosemary and turmeric) against bacterial isolates was also evaluated.
Methods: A total of 20 beef samples were obtained from open markets and malls in Accra and microorganisms were enumerated using standard microbiological methods. Bacteria were subjected to susceptibility testing to cefepime (30µg), gentamicin (10µg), tetracycline (30µg), meropenem (10µg), and nalidixic acid (5µg). Turmeric, rosemary oils and mixtures of both as potential antimicrobial agents were tested against selected microorganisms (*E. coli, Salmonella* spp., *Shigella* spp., *Proteus mirabilis and Klebsiella pneumonia*).
Results: Samples of raw beef from both markets revealed the prevalence of *Enterobacteriaceae*, predominantly *Escherichia coli* (20%), followed by *Proteus mirabilis* (14%), *Salmonella spp*. (11%), *Shigella sonnei* (5%) and *Klebsiella pneumonia* (2%). *Enterobacter spp.* (5%) and Citrobacter *fruendi* (7%) were isolated only from open market samples, while *Morganella morganii* (2%), *Providencia spp.* (2%) and *Shigella spp.* (2%), were isolated from mall samples. Meropenem (10µg) and Gentamycin (10µg) showed activity against a number of *Enterobacteriaceae* isolated from raw beef samples, except for *Salmonella* and *Escherichia coli* species. *Morganella morganii, E. coli O157:H7, Providencia spp.* and *Salmonella spp.* showed a significantly higher resistance to the antibiotics. Rosemary was effective against *Escherichia coli* and *Proteus mirabilis* at an MIC of 10%, respectively. Turmeric, on the other hand, showed efficacy against *Salmonella* spp., *Shigella* spp. and *Klebsiella pneumonia* at MICs of 5%, 10%, and 10%, respectively.
Significance: Raw beef from the open market was found to be highly contaminated with bacteria and fungi, therefore hygiene checks at points of sale should be regular and retailers should be educated on safe food handling.

P2-55 Risk Assessment of Mycotoxin Exposure in Complementary Foods for Children aged 6 Months to 2 Years in Accra, Ghana

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Introduction: Infants and young children are given complementary foods as a supplement to breast milk and/or formula. Mycotoxins in the environment often contaminate food crops such as cereals, grains and nuts.
Purpose: This study was conducted to assess the risk associated with the intake of mycotoxin residues, i.e., aflatoxin, ochratoxin A, and fumonisin B1 through the consumption of complementary foods obtained from markets and shopping centers in Accra, Ghana.
Methods: About seventy (70) samples of complementary foods were collected and HPLC analysis was performed to determine the levels of mycotoxins (total aflatoxin, aflatoxin B1, ochratoxin A and fumonisin B1). In addition, this study included a consumer survey that collected information from 392 participants on the consumption of complementary foods among children aged 6 months to 2 years in Accra.
Results: From the survey, 58.4% of the children were female. The majority (43.1%) were between 6 and 9 months of age and 50% of them weighed between 8 and 10 kg. Also, 43.1% of children received complementary food at least once a day. About 4.3% (aged 13-24 months) were fed complementary food at least four times a day. The average concentration of aflatoxin, ochratoxin A and fumonisin B1 was 45.68 µg/kg, 11.2 µg/kg and 4.98 mg/kg, respectively. It was found that the concentration of aflatoxins was significantly higher than the regulatory limit (5µg/kg). The concentrations of ochratoxin A and fumonisin B1 were within the regulatory limits (20 µg/kg and 4 mg/kg, respectively). In general, the health risk associated with dietary exposure to mycotoxins in complementary foods, particularly aflatoxin, was substantial.
Significance: The presence of these mycotoxins in complementary foods is a major public health and food safety concern. Market surveillance, product testing and enforcement of regulatory laws should be intensified to protect children's health from the negative consequences of dietary exposure to mycotoxins.

P2-56 Microbial Diversity and Antimicrobial Resistance in Small Scale Goat and Sheep Farms

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Introduction: The emergence of Antibiotic-resistant bacteria (ARB) in zoonotic pathogens presents a significant threat to both animal production and human health. While large-scale animal farms are recognized as significant reservoirs for AMR, there remains a notable knowledge gap regarding AMR in small-scale farms.
Purpose: This study aims to assess the microbial diversity and antimicrobial resistance of bacteria in small-scale goat and sheep farms through the analysis of fecal samples.
Methods: A total of 137 fecal samples were collected from goat and sheep farms. located in Tennessee and Georgia. Samples were analyzed using culture-dependent method and 16s rRNA amplicon sequencing. Additionally, Antimicrobial Susceptibility Testing (AST) was performed using the Kirby-Bauer Disk Diffusion method to assess the susceptibility of bacteria to various antibiotics.
Results: The dominant phyla in goat and sheep feces were Firmicutes, Bacteroidetes, Proteobacteria, and Spirochaetes. The α-diversity indices indicated a stable microbial diversity across diverse sample types and farm locations. Conversely, β-diversity analysis unveiled significant disparities in microbial community composition contingent upon both sample type and farm location. The prevalence of *E. coli* (94.9%), *S. aureus* (91.3 %), and *S. saprophyticus* (81.0%) were markedly higher ($p < 0.05$) than that of *Shigella* spp. (35.0%) and *Salmonella* spp. (3.0%). The antibiotic resistance was high against ampicillin (79.4%), vancomycin (65.1%), and gentamycin (63.6%), significantly surpassing ($p<0.05$) resistance to tetracycline (41.6%) and imipenem (21.8%). Moreover, the penicillin (79.4%), glycopeptide (65.1%), and aminoglycoside (63.6%) antibiotic classes exhibited significantly higher ($p < 0.05$) resistance compared to tetracyclines (45.7%) and carbapenem (21.8%).
Significance: The presence of ARB in the feces of goats and sheep underscores significant ecological risks, particularly within farm environments. ARB present in feces can potentially spread into soil, contaminate water sources, and ultimately enter the food chain. Understanding the prevalence of ARB in farm settings enables producers to make informed decisions regarding disease control measures, antibiotic usage practices, and the promotion of sustainable agriculture.

P2-57 Exploring Antimicrobial Synergies to Combat *Burkholderia cepacia* Biofilm Formation

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❖ ***Developing Scientist Entrant***

Introduction: Food spoilage is a major concern in the food industry. The ability of microorganisms to produce biofilm on the surface of food processing equipment is major cause of spoilage. *Burkholderia cepacia* is an underexplored pathogen in the food context, but the bacterium serves as an ideal model for biofilm formation.
Purpose: To explore potent antimicrobial compounds that act synergistically against *B. cepacia* biofilm
Method: *Burkholderia cepacia* ATCC 25416 was assessed for its ability to produce biofilm in three different microbiological media: Tryptic Soy Broth

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(TSB), Yeast Dextrose Calcium Carbonate (YDC), and Luria Bertani (LB). The bacterium was grown under aerobic conditions at 37°C for 24 and 48h in a 96-well microtiter plate before quantifying the adhering biofilm to the surface of the wells using crystal violet biofilm assay. The minimum inhibitory concentration (MIC) of various antimicrobial agents was determined using broth microdilution method and the ability of these agents to inhibit initial biofilm formation was determined. The antimicrobials with the lowest MIC values were then tested for their synergistic effects against *B. cepacia* biofilm using checkerboard technique. Data were analysed using statistical software JMP 17 Pro and significance was determined at $p < 0.05$.

Result: The use of YDC media and incubation for 24h were the optimum conditions for inducing biofilm formation by *B. cepacia*. Ciprofloxacin, ceftazidime, and meropenem trihydrate, showed MIC values of 0.4, 10.0, and 7.0 ug/ml, respectively. These antimicrobial agents exhibited antibiofilm activity by reducing initial attachment of *B. cepacia* biofilm cells. Four antimicrobial combinations at various concentrations (sublethal to lethal) were tested using the checkerboard method, and results indicated that the combination of ciprofloxacin and meropenem trihydrate exerted the best synergism against *B. cepacia* biofilm.

Significance: Developing synergistic antimicrobial combination holds promise as alternative to conventional antimicrobial treatment in preventing *B. cepacia* biofilm formation and toxin production in food.

P2-58 *In vitro* Biofilm Inhibition, Anti-Inflammatory, and Cationic Dye Reduction Activity by Hydrothermal Assisted Citric Acid-Doped Carbon Quantum Dots from Walnut Shell Biomass

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Introduction: The valorization of plant-based biomass into carbon quantum dots (CQDs) is of prime significance to green chemistry. Walnut shell is one of the non-toxic waste materials consisting of high carbon content that has been used for the synthesis of CQDs hydrothermally. However, the doping of these CQDs with citric acid to produce highly luminescent nanoparticles under mild conditions has not yet been achieved.

Purpose: This study aimed at the green synthesis of citric acid-doped CQDs derived from walnut shell and studied its characteristics. Moreover, we investigated the biological activities of CQDs.

Methods: CQDs were prepared from walnut shell biomass using the green hydrothermal method using citric acid as a precursor. The synthesized CQDs were characterized using transmission electron microscopy (TEM), UV-visible spectroscopy, and fluorescence spectroscopy. The cytotoxicity, antimicrobial, anti-biofilm, anti-inflammatory, and dye reduction properties of CQDs were determined using standard methodologies.

Results: The prepared CQDs exhibited 270 nm absorbance and 440 nm fluorescence under the excitation of 350 nm light. Semi-spherical CQDs with a size less than 6.08 nm were obtained. The cytotoxicity test suggested that prepared CQDs showed cell viability of 99.04% and anti-inflammatory activity of 72.92%. The susceptibility of *Salmonella typhimurium*, *Escherichia coli*, and *Pseudomonas aeruginosa* was found to be higher than that of *Listeria monocytogenes*, with a minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 500 µL/mL and 1000 µL/mL, respectively. Additionally, prepared CQDs exhibited a higher killing rate and lower antibiofilm activity percentage for *S. typhimurium*, *E. coli*, and *P. aeruginosa* compared to *L. monocytogenes*. The prepared CQDs exhibited a remarkable 96.45% degradation efficiency of safranine dye under visible light.

Significance: The synthesized CQDs are promising materials for the functioning of bioimaging, pharmaceuticals, food safety and food industry applications.

P2-59 Impact of Undissociated Weak Acid Concentration on the Growth Kinetics of Fungal Species in White Bread

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Introduction: pH plays an important role in the effectiveness of calcium propionate, a commonly used preservation system in bakery products, for mold inhibition.

Purpose: Assess the effectiveness of calcium propionate (ProBake) and lactic acid as preservation system in inhibiting mold growth.

Results: The interaction between calcium propionate and lactic acid influenced the pH levels of the treatments. The pH values were 5.49 for the negative control, 5.34 for the treatment with 0.4% ProBake and 5.26 for treatment with 0.4% ProBake and 0.1% lactic acid. Mold growth was observed in all negative control samples at day 4. Samples containing 0.4% ProBake and inoculated with *A. niger, P. chrysogenum* and *P. commune*, significantly increased days-to-mold compared to control at 12, 12, and 5 days, respectively. Similar trends were observed for 0.4% ProBake and 0.1% lactic acid, further extending the shelf of the bread with mold appearing at day 7 (*P. commune*), 18 (*A. niger*), and >29 (*P. chrysogenum*). However, for *Penicillium roqueforti*, both formulations molded at day 5.

Materials: Loaves of white bread were used to investigate the impact of pH on mold growth. Three types of loaves were prepared: no preservation system (negative control) and the others with 0.4 % calcium propionate (ProBake) along with varying concentrations of lactic acid (0 and 0.1%). The pH levels of the breads were measured on day 0. Loaves were inoculated with four different mold species: *Aspergillus niger*, *Penicillium commune*, *Penicillium roqueforti*, and *Penicillium chrysogenum*. The bread loaves were sealed in plastic bags and stored at 20°C for 29 days. Bread surface was observed daily to monitor the mold growth. Differences among the treatments were determined using one-way ANOVA at $p<0.05$.

Significance: The combination of low pH and weak acid preservatives play an important role in influencing the behavior of mold species, serving as a key microbial safety indicator.

P2-60 Genomic Analysis Identifies Plasmid-Borne Biosynthetic Gene Clusters with Potential Antimicrobial Products in Actinomycetes

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Introduction: Actinomycetes are a group of Gram-positive bacteria that produce secondary metabolites (e.g. antimicrobials) with potential use in the food industry to control the growth and survival of foodborne pathogens. Investigation of actinomycete carrying gene clusters can lead to the identification of new antimicrobials with broad host range and activity.

Purpose: Biosynthetic gene clusters (BGCs) in actinomycete plasmids that encode putative antimicrobial compounds were assessed.

Methods: Actinomycete genomes (n=66,379) were retrieved from NCBI and screened for plasmids using the MOB-suite pipeline. 14,773 plasmids were identified and screened for BGCs using antiSMASH (v7.1). BGCs were clustered using the BiG-SCAPE (v1.1.5) pipeline. Data were cleaned and putative BCGs were identified by determining the closest homology to known BGCs using the MIBiG database. Annotation files of clustered BGCs and their gene structure were examined for genes encoding putative antimicrobial compounds.

Results: 1491 actinomycete plasmids had one or more BGC. 101 unique plasmids were selected for further analysis through data filtering steps. These 101 plasmids were from 15 genera including *Streptomyces* (n=40), *Mycobacterium* (n=12) and *Rhodococcus* (n=12). Using BiG-SCAPE, 129 unique clusters were identified from those plasmids, which include polyketide synthases (n=46), ribosomally synthesized and post-translationally modified peptides (n=31), nonribosomal peptide synthetases (n=26), "other" (n=20), terpenes (n=5), and saccharides (n=1). Of note, 84/129 of the BGCs detected had percentage similarity scores that ranged from 3 – 100% to known BGCs. So far, some clusters (n=16) have been identified, including the most similar cluster identified in *Pseudonocardia* sp. (HH130630-07 plasmid pLS2-2), encode products similar to antimicrobials, some of which could be used to control foodborne pathogens, including *Listeria monocytogenes* and *Bacillus* spp.

Significance: Preliminary results suggest some BGCs on actinomycete plasmids have potential antimicrobial activities. Further investigation into BGC product properties would determine their effectiveness in the control of foodborne pathogens and other potential applications in the food industry.

P2-61 Evaluation of Synergistic Bactericidal Activity of Nanobubbles and Peracetic Acid and the Underlying Mechanism

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Introduction: US FDA recommends treating irrigation water with antimicrobial agents to successfully mitigate microbial risk. Nanobubbles (NBs) are stable, high-surface-area bubbles that, upon physical rupture, can produce antimicrobial activity. However, the efficacy of nanobubbles with antimicrobials to sanitize irrigation water, along with their microbial inactivation kinetics and underlying mechanisms, has been scarcely explored.
Purpose: To evaluate the combined impact of bulk nanobubbles and peracetic acid (PAA) on *Escherichia coli* TVS 353 and *Listeria innocua, and* to understand the kinetics and mechanisms of inactivation.
Method: Three flasks each containing 98 mL of distilled water (control), 99 mL of PAA solution (4 ppm, S) and NBs sparged water with PAA solution (4ppm, NS) were inoculated with 1 ml of *E. coli* TVS 353 and *L. innocua* (each log 8 CFU/mL) . Samples were drawn at intervals of 1 minute for 6 minutes and were immediately transferred into neutralizer solution (0.28 g/ml sodium metabisulfite) for further bacterial enumeration. The mechanisms of inactivation were assessed using a) membrane damage using the propidium iodide assay; b) oxidative damage using the CellRox green reagent; and c) metabolic activity using the resazurin assay. All the experiments were done in five replicates, and statistical analysis was performed using ANOVA and Tukey's HSD test ($P<0.05$; at 95% confidence level).
Results: The significant increase in bacterial inactivation was triggered by the combined effect of NBs and sanitizer, leading to a difference of 1 log survivor after 6 minutes of treatment. The average D-values (decimal reduction time) for *E. coli* TVS 353 sanitized in PAA solution with and without NBs are 116±12 s and 192±15 s, respectively, and for *L. innocua*, 120±5 s (PAA with NBs) and 170±13 s (PAA without NBs). The inactivation of both microorganisms followed the Weibull kinetic model with a characteristic shoulder.
Significance: This study illustrates the potential of applying nanobubbles with PAA as alternative, novel methods for successfully sanitizing irrigation water.

P2-62 Turmeric as a Food-compatible Photosensitizer to Improve the Safety of Minimally Processed Kale Pesto

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Introduction: Novel methods for decontaminating food matrices, such as photodynamic inactivation (PDI) using photosensitizers (PSs), have shown promise for pathogen control. Food-compatible PSs should not affect the composition, taste, or flavor of food. We propose turmeric as a source of well-regarded Ps curcumin to mitigate foodborne pathogens in minimally processed leafy vegetable products.
Purpose: A combination of turmeric and blue light (BL) was investigated to eradicate *Listeria monocytogenes* (3-strain cocktail: 10^6 CFU) in a sous-vide kale pesto. We have developed a pesto recipe that is minimally processed and checked for treatment efficacy against *L. monocytogenes* and changes in color.
Methods: 8% (w/w) turmeric was added to the pesto, which was then subjected to overnight exposure to BL (410-460 nm, 65 mW/cm²) and sous-vide (S-V) cooking at 60 °C for 8 min. Viability was measured through the plate count method. Color measurements were obtained through instrumental analysis and assessed using the CIE L*a*b* system.
Results: S-V and BL reduced *L. monocytogenes* by 1.7 logs, while adding turmeric increased the treatment efficacy by an additional 2.0 logs, decreasing *L. monocytogenes* from 6.0 to 2.3 log CFU/g ($p<0.05$). In addition, S-V alone and S-V with turmeric caused 0.6 and 1.8 log cell reductions, respectively, suggesting turmeric activation at 60 °C. After treatment, plain pesto showed a considerable decrease in lightness (L*) ($p<0.05$), while pesto with turmeric displayed only a slight decrease ($p>0.05$). The greenness (a*) remained unaffected by turmeric, while the yellowness (b*) increased slightly ($p>0.05$). The treatment caused both pesto products to undergo similar color changes, becoming less green and yellow ($p<0.05$).
Significance: In conclusion, by incorporating turmeric into PDI, we can maximize its effectiveness while maintaining safety and compatibility with food products.

P2-63 Expansion of the National Antimicrobial Resistance Monitoring System (NARMS)'s Retail Meat Testing Program Through the FDA's Laboratory Flexible Funding Model (LFFM), July 2022 to June 2023

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Introduction: Over the last two decades, the National Antimicrobial Resistance Monitoring System (NARMS)'s retail meat surveillance has expanded significantly, but geographic data gaps persist, despite efforts to ensure representation from all states.
Purpose*:* This study aimed to expand the NARMS retail meat surveillance to additional states using the FDA's Laboratory Flexible Funding Model (LFFM) Cooperative Agreement Program, which enabled the collaboration with nine state public health, agricultural, and academic laboratories (California, Indiana, Iowa, Michigan, New Hampshire, New Jersey, Ohio, Virginia, and West Virginia).
Methods*:* From July 2022 to June 2023, the nine laboratories performed routine retail meat sample collection following a stratified random sampling plan. Standardized isolation protocols from NARMS, FDA's *Bacteriological Analytical Manual* (BAM), or USDA's *Microbiology Laboratory Guidebook* were used. All laboratories tested for *Salmonella* in retail pork and beef samples and two laboratories (Ohio and Virginia) also tested for *Campylobacter* in retail chicken samples. Confirmed isolates were shipped to FDA's Center for Veterinary Medicine for antimicrobial susceptibility testing. Whole-genome sequences were submitted to the National Center for Biotechnology Information under NARMS or GenomeTrakr BioProjects.
Results*:* A total of 924 retail packages were collected and 1,154 analyses performed. These resulted in 85 positive samples that yielded 113 *Salmonella* and 38 *Campylobacter* isolates. Prevalence data mirrored previous years of NARMS retail meat surveillance data (*Salmonella*, 1-2% in beef, 2-4% in pork, 15-22% in chicken; *Campylobacter*, 9-13%). Whole-genome sequencing data submitted allowed for national and global monitoring of antimicrobial resistance trends and determinants.
Significance: The success of this unique collaboration between FDA and states has allowed this hallmark antimicrobial resistance surveillance program to reach 30 states for retail meat testing, filling important data gaps. The continuation of this collaboration into future years supports the expansion of NARMS to protect public health under the One Health framework.

P2-64 Development of Chlorinated Zein-Coated Beads for Water Disinfection

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Introduction: Water treated with chlorination for antimicrobial disinfection often contains free chlorine, such as NaClO and HClO, which can react with organic compounds and form carcinogenic disinfection by-products (DBPs) on fruits and vegetables. Zein is a storage protein commonly found in corn.
Purpose: To mitigate the production of DBPs, an antimicrobial water disinfection system was developed using plastic beads coated with corn-derived protein zein.
Methods: 0.125 inch diameter high-density polyethylene (HDPE) plastic beads were immersed into 3% zein solution (w/v) dissolved in 80% ethanol (v/v) and dried completely in a food dehydrator. The resulting beads underwent chlorination with a 1% bleach solution at pH 5.0-6.0 and were subsequently rinsed with deionized water to get rid of excess free chlorine. The beads were assessed for the total chlorine retained within the zein coating through total chlorine titration after washing. Additionally, the study investigated the long-term storage stability of retained chlorine under 4°C and 25°C and assessed its antimicrobial efficacy in deionized water containing *Escherichia coli* or *Listeria innocua*.
Results: Based on studies using total chlorination titration, the optimal formulation was determined to include a diameter size of 0.125 inches, HDPE material, and a 3% (w/v) coating solution with 107.54 ± 8.9 µg of chlorine retained with three beads. Beads stored at 4°C demonstrated higher total chlorine retention on day 28 (63.81 ± 2.1 µg) compared to those stored at 25°C (50.1 ± 6.4 µg). Lastly, the three beads exhibited significant antimicrobial activity, achieving a 5-log reduction in *Escherichia coli* and a 4-log reduction in *Listeria innocua*.
Significance: Based on results, chlorinated zein-coated beads have shown success in being utilized as a potential antimicrobial disinfection system for water. This disinfection system has the potential to remove pathogenic bacteria from agricultural water while minimizing the production of DBPs.

P2-65 A Technical Evaluation of Two Commercially Available Natural Mold Inhibitors for Their Ability to Extend the Shelf Life of Cheese

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Introduction: The most common molds that contaminate cheese are *Aspergillus* and *Penicillium* species, which can grow in a wide temperature range and produce mycotoxins that are a threat to human health.
Purpose: Extend shelf-life and prevent mold spoilage of cheese with natural antimicrobials such as cultured dextrose and cultured skim milk in comparison to natamycin.
Methods: Cheddar and mozzarella cheese squares of 25-35g were inoculated with *Penicillium crustosum* FRR 1809 and *Aspergillus flavus* aflatoxin producer (B_1, B_2) and sprayed with 1% cultured dextrose, 1% cultured skim milk, or 15 ppm natamycin. The treatments included a control and a spiked control with no antimicrobial. The samples were held at 4°C and 10°C, and analyzed for water activity (a_w), pH, moisture, ash, protein, lipids, and carbohydrates at Day 0 and throughout the study before visual mold was detected. Shelf life was analyzed with a mixed-model split-plot analysis in a 2x2x5 factorial experiment in triplicates. Storage temperature was the whole plot treatment and three random replications for each temperature where cheese type (C: cheddar or M: mozzarella), mold type (A: *Aspergillus flavus* and P: *Penicillium crustosum*), and sample type (C: control, N: Natamycin, N1: Cultured dextrose, N2: cultured skim milk, SP: spiked).
Results: The most significant interaction was between mold and antimicrobial applied; for instance, natamycin and cultured dextrose were equally effective in controlling *Aspergillus flavus* in both cheeses and extended the shelf life by 23-25 days. However, natamycin inhibited *Penicillium crustosum* better than cultured dextrose or cultured skim milk. Based on the active ingredient in natural antimicrobial, the use level can be adjusted to prevent mold and achieve desired shelf life.
Significance: Having different mold inhibitory solutions, the cheese industry can meet the consumer demand for clean label and natural solutions while minimizing food waste, consumer complaints, and avoid potential recalls due to mold contamination.

P2-66 Effect of Clove Oil in Reducing Aflatoxin B1(AFB1) in Organic Peanuts infected by *Aspergillus flavus* in Georgia

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Introduction: Peanut crops infected by Aspergillus species continues to be a problem in Georgia (1) and there are no direct-action peanut farmers can take to control aflatoxigenic fungi in peanut (2).
Purpose: In our previous study, we reported that Clove Oil showed the maximum anti-fungal and anti-aflatoxigenic activities against *A. flavus* (3) and in this study, we evaluated the effect of Clove EO in reducing AFB1 in organic seeds.
Methods: 100 seeds, variety GA Greener, were artificially inoculated with *A. flavus* (10^6 /mL) and incubated at 28°C for 8 days. Treated seeds were exposed to Clove EO at concentrations 0, 500, 1000, 1500, and 2000 ppm, respectively. Treated seeds (5/plate) were then placed equidistantly in Petri plates with moist filter paper and incubated similarly, with alternate light and darkness. Tween 20 (0.05%) served as control (4) and all experiments were done in replicates. AFB1 quantification was done by Ion Monitoring (SIM) based Liquid Chromatography and Mass Spectrometry (LCMS). AFB1 standard was used for comparison. A simple t-test was done for significance of data.
Results: UV spectrum of AFB1 reference standard was identical to that of Clove EO treated samples confirming the presence of AFB1 in the seed extracts. A significant reduction in AFB1 production was seen between 0 and 500 ppm of Clove EO, and the reduction slightly increased with higher concentrations of 1000 and 2000 ppm compared to the control. This demonstrates that Clove oil appeared to be most effective, at low concentrations, in reducing AFB1 in contaminated peanuts. Future studies will be done in Vivo.
Significance: Our findings provide evidence of potential use of Clove Oil as an eco-friendly alternative to synthetic fungicides against *A. flavus* in peanuts and Integrated Pest management (IPM) program.

P2-67 Identification of Broad-Spectrum Bacteriocins as Potential Biopreservatives for Use in Foods and Identification of Their Structural Genes

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❖ ***Developing Scientist Entrant***

Introduction: Bacteriocins are antimicrobial peptides produced by Bac+ bacteria. Those produced by lactic acid bacteria (LAB) often contribute as natural preservatives against food spoilage and pathogenic bacteria.
Purpose: The purpose of this study was to isolate and characterize broad-spectrum bacteriocins that might have applicability as biopreservatives in food and to identify their gene sequences.
Methods: Bac$^+$ isolates were obtained from retail foods by testing against pathogenic indicator strains by colony overlay, spot-on-lawn, and indicator patch plate methods. Identities were obtained by PCR of 16S rRNA, sequencing, and BLAST search of NCBI databases. Primer pairs of known bacteriocin genes were used for amplification and direct sequencing, or after blunt-end cloning of Bac-gene amplimers, to identify bacteriocin structural gene sequences.
Results: We isolated broad-spectrum Bac$^+$ LAB and successfully sequenced several bacteriocin-related structural genes using the bacteriocin PCR array

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without any prior sequence information of the isolates. Amplimers were obtained from 2 different Bac+ isolates (*Lactiplantibacillus plantarum, Leuconostoc mesenteroides*) with the same set of primers from plantaricin A (*pln*A). Both strains demonstrated broad spectrum inhibitory activity against multiple indicator organisms, notably *Clostridium* perfringens. This was the first identification of plantaricin gene sequences found outside of *L. plantarum* and the first found in *Leuconostoc spp.* We further identified Bac+ spore-forming *Bacillus* spp. (*Bacillus subtilis, Bacillus halotolerans, Bacillus thuringiensis*) which may expand the application of Bac+ probiotic biopreservatives via spore-forming *Bacillus spp.* in the food industry.

Significance: This was the first identification of plantaricin outside of *L. plantarum* (i.e., in *Leuconostoc*). Genetic sequences of bacteriocin structural genes may provide insights into the biochemical mode of action of these antimicrobial peptides. The identification of broad-spectrum pathogen-inhibiting bacteriocins could be useful as biopreservatives and/or probiotics in foods; those inhibiting *Clostridium* spp. could mitigate or compliment the use of nitrite in processed meats.

P2-68 Characterization and Screening for the Potential Target against Biocide Resistance among Foodborne Bacteria

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Introduction: Foodborne pathogens like *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* (STEC) encounter antimicrobials throughout the farm-to-fork continuum. Wildlife interactions with livestock at the farm level play a crucial role in antimicrobial resistance (AMR) ecology, including resistance to common biocides like Triclosan (TRI) and Benzalkonium Chloride (BZC). Widespread biocide use raises concerns about reduced susceptibility and cross-resistance to antibiotics, leading to the selection and spread of multi-drug resistant pathogens. Resistance often emerges during food processing and/or in the environment, facilitated by mechanisms like efflux pumps (EPs).

Purpose*:* Characterization and screening for the potential target against biocide resistance among bacterial isolates from retail meat (RM) and wildlife (WL) via *in-vitro* and *in-silico* approaches.

Methods*:* Isolation of bacterial isolates from RM (turkey, beef, chicken, pork) and WL using culture-based method, further species-level identification by MALDI-TOF. The confirmed isolates of *E. coli* and *S. enterica* were then subjected to antimicrobial and biocide susceptibility testing (BST). Docking study was also conducted to understand the drug-protein interactions between EP and biocides.

Results*:* Examining 100 samples each from RM and WL confirmed antibiotic resistance: 82.3% *E. coli* and 85.1% *Salmonella* in RM, highly resistant to tetracycline. Previously isolated resistant WL *E. coli* was analyzed for BST showed 64.6% resistance to TRI with susceptibility to BZC. In RM, 56.7% and 57.4% resistance of *E. coli* and *Salmonella* respectively was observed against TRI and 5.6% *E. coli* with no *Salmonella* resistance was found against BZC. The docking study with EP protein revealed interactions with TRI and BZC, yielding binding energies of -8.13 kcal/mol and -7.62 kcal/mol, respectively, suggesting EP as a promising target to address biocide resistance.

Significance: The observed resistance trends and docking data emphasize the need for a detailed exploration of EP as a target to combat resistance. The results underscore the importance of integrated public health approaches and enhanced food safety practices across both the food industry and wildlife settings.

P2-69 Prophylactic Efficacy of Peppermint Essential Oil to Inhibit Biofilm of the Foodborne and Food Spoilage Pathogens

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Introduction: Establishing efficient methods to combat bacterial biofilms is a major concern. Natural compounds, such as essential oils derived from plants, are among the favored and recommended strategies for combatting bacteria and their biofilm.

Purpose: Therefore, we evaluated the antibiofilm properties of peppermint oil as well as the activities by which it kills bacteria generally and particularly their biofilms. Peppermint oil antagonistic activities were investigated against *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium on four food contact surfaces (stainless-steel, rubber, high-density polyethylene, and polyethylene terephthalate).

Methods: Biofilm formation on each studied surface, hydrophobicity, auto-aggregation, metabolic activity, and ATP quantification were evaluated for each bacterium in the presence and absence (control) of peppermint oil. Real-time PCR, confocal laser scanning microscopy, and field emission-scanning electron microscopy were utilized to analyze the effects of peppermint oil treatment on the bacteria and their biofilm.

Results: Results showed that peppermint oil (1/2× MIC, MIC, and 2× MIC) substantially lessened biofilm formation, with high bactericidal properties. A minimum of 2.5 log to a maximum of around 5 log reduction was attained, with the highest sensitivity shown by *V. parahaemolyticus*. Morphological experiments revealed degradation of the biofilm structure, followed by some dead cells with broken membranes. Thus, this study established the possibility of using peppermint oil to combat key foodborne and food spoilage pathogens in the food processing environment.

Significance: From these results, we suggest that PEO may contribute to improving food quality by lowering bacterial contamination on food contact surfaces.

P2-70 The Effects of Antimicrobial Coating and Acid Washes on Microbial Growth during Mung Bean Seed Germination and Sprouting

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Introduction: Sprouts geminate best in warm, humid conditions, favoring growth of foodborne pathogens, such as enterohemorrhagic *E. coli*, *Listeria monocytogenes* or *Salmonella,* and can lead to illness, as they are often consumed raw or lightly cooked. Contaminated sprouts have been implicated in numerous foodborne illness outbreaks and product recalls. Antimicrobial coatings and acid washing solutions have been widely studied to reduce microorganisms on fresh produce.

Purpose: This study investigated the efficacy of antimicrobial coating+acid washing to inactivate microorganism on mung bean sprouts.

Methods: Seeds (100 g) were inoculated with *L. innocua* or *E. coli* at approximately 10^5 CFU/g, dried for 2 h, then coated with a solution of 0.5% chitosan+0.5% acetic, lactic and levulinic acids. The seeds germinated at 22°C for 5 days. Twice a day, seeds were soaked in a 0.5% acetic acid solution for 1 min. Sterile tap water was used as a control and the following four treatments were investigated: T1: coating+acid washing; T2: coating+water washing; C1: acid washing; and C2: water washing. Uninoculated seeds were used for background microbiota and sprout gemination tests. The experiments were conducted three times and data were analyzed by SAS software.

Results: Populations of *E. coli* and *Listeria* were significantly lower ($p<0.05$) following T1 treatment, achieving 2.0 log/g reduction of *E. coli* and 4.0 log/g reduction of *Listeria* after 1 day. Although both populations on all samples increased during the 5 days' gemination, their numbers were lowest in T1 samples, followed by T2, C1 and C2, at time of harvest. Similarly, T1 treatment reduced 3.3 log/g of total plate counts and 3.0 log/g of yeast and mold counts. There were no significant differences ($p>0.05$) in germination rate and weight gain among all treatments.

Significance: These results demonstrate a new approach to reducing microbial contaminants while growing sprouts, without negatively impacting germination rate or weight gain. Future studies will be conducted to improve the antimicrobial efficacy.

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P2-71 Microbiological Effects of Peroxyacetic Acid Spray for Beef Carcasses and Cuts Under Laboratory and Commercial Settings

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Introduction: Treating beef carcasses and cuts with peroxyacetic acid (PAA) has gained more popularity recently in Canadian meat processing facilities. However, the microbiological effects of PAA varied greatly between different studies.
Purpose: To determine and validate conditions affecting the effectiveness of PAA spray for beef under laboratory and commercial settings.
Methods: Inactivation of *E. coli* by up to 400 ppm PAA in low (100 mg/L) and high (1350 mg/L) organic load solutions was determined. Microbiological effects were assessed for 400 ppm PAA spray for beef plates (400 cm^2/side) inoculated with cattle fecal slurry after PAA spray (n=54) or during the subsequent storage at 1°C for up to 7 days (n=84). The microbial efficacy of PAA was further validated at two commercial beef plants for carcasses and cuts. Least-squares means of the log reduction values were separated using the Tukey test.
Results: *E. coli* was reduced by > 7 log CFU by exposure to PAA at >100 ppm and 200 ppm in low and high organic load solutions. Spraying beef plates with 400 ppm PAA reduced coliforms and *E. coli* by 1.0-1.2 log units at room temperature and 1°C, compared to water spray ($p<0.05$), while no significant changes were noted during subsequent storage. PAA spray reduced ($p<0.05$) coliforms and *E. coli* by 1.7-2.0 log units on carcasses artificially inoculated with fecal slurry at one plant, and aerobes and coliforms by 1.7 and 1.0 log units on naturally contaminated carcasses at the other meat plant ($p<0.05$). Significant and consistent reduction of aerobes and coliforms on fat surface cuts by PAA was observed at both meat plants.
Significance: PAA spray can be an effective antimicrobial intervention for controlling enteric pathogens on beef carcasses and cuts and may be beneficial for reducing spoilage microorganisms.

P2-72 Rapid Screening of Microorganisms from Ultra-High Temperature (UHT) and Extended Shelf-life (ESL) and Acidic Drinks Using Hygiena's Innovate™ System

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Introduction: Aseptic processing is a widely used method in food and beverage processing. Traditional methods for microbial testing can take several days to weeks for results. The InnovateTM Rapid Microbial Screening System utilizing the RapiScreenTM Beverage kit can analyze adenosine triphosphate (ATP) bioluminescence for rapid microbial screening of UHT, ESL, and highly acidic drinks, providing rapid results.
Purpose: The purpose of this study was to demonstrate the rapid detection of six different organisms spiked into 6 different product matrices, ranging from dairy to plant-based to fruit-flavored matrices.
Methods: For each product matrix, 20 replicate test portions were spiked at a fractional level of inoculation with *B.cereus, B.subtilis, C.sporogenes, L.fermentum, P.aglomerans,* and *S.cerevisiae* to achieve 5-15 positive results out of 20 tested. On each sampling day (Day 1,2,3,5 and 7), 50 µL aliquots of enriched product were transferred to the Innovate System plate. Confirmation plates were prepared to confirm the growth of each target organism and pour plates were performed on day 15 in accordance with the ISO 4833:1:2012 method.
Results: Testing of six different matrices showed that the probability of detection for the Innovate RapiScreen Beverage kit was at 100% for high and low inoculation levels when compared to the plating method. The kit delivered detection of contaminated product packs in 7 days or less with results that are equivalent to the 15-day reference method requirement.
Significance: Based on this study, we can conclude that the Innovate System using the RapiScreen Beverage kit detected microbial contaminants in the examined matrices at least 10 days quicker than the reference method.

P2-73 Impact of pH and Incubation Temperature on Hold Time Requirements of Acidified Sauces to Comply with US-FDA's Acidified Foods Regulations

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Introduction: Acidified Foods regulations of FDA (21 CFR 114) is a complex subject for manufacturers of shelf-stable sauces, condiments and dressings not only in the U.S. but also in other parts of the world exporting their products to the U.S. Apart from satisfying FDA's process filing requirements, manufacturers also need to demonstrate the safety and stability of these products through challenge tests resulting in mandatory hold time requirements, if the products are cold filled.
Purpose: The objective of this study is to evaluate the effect of different pH and incubation temperatures on hold time requirements to demonstrate a 5-log reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella*.
Methods: Acidified sauce samples with adjusted equilibrium pH ranging from 3.7 to 4.2 were inoculated with acid-adapted pathogens at 10^6-10^7 log levels. Along with controls, these were incubated at various temperatures (10, 18 and 25ºC) representing various storage, shipping and distribution conditions. Aliquots of samples were plated at frequent intervals to determine pathogen counts using BAM methods.
Results: Among the pathogens tested, *E. coli* O157:H7 was found to take the longest to achieve the required five-log reduction (3 days to >15 days). The time to achieve five-log reduction was longer for products with higher pH and for products that are incubated at lower temperatures.
Significance: The study demonstrated the need for critical control of pH at lower levels and possibly storing the sauces at higher temperatures or ambient conditions to reduce the mandatory hold time required for achieving 5-log reduction of pathogens before releasing the product to market. This hold time and incubation temperature serves as the scheduled process in FDA's filing, which the manufacturers exporting their products should be aware of.

P2-74 Validation of In-Pack Pasteurization of Fresh-Pack Pickles in a Continuous Flow Pasteurizer Tunnel

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Introduction: Fresh whole dill cucumbers placed in weak brine is thermally processed for safety and stability. While elevated thermal process conditions ensure stability against spoilage it may adversely affect the product in terms of firmness and flavor. It is necessary for the manufacturer to validate and demonstrate the process systems in-place for consistent and effective delivery of lethality against acid-forming bacteria and yeasts as a minimum.
Purpose: The objective of this study is to validate the continuous pasteurizer tunnel and establish the process parameters to deliver lethality against the FDA's requirement of 5-log reduction of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella*.
Methods: Validation was conducted on six tightly packed cans (#10 size) of small dill cucumbers stressed with increased product weight and reduced headspace. Thermal distribution and heat penetration studies were conducted at pre-set pasteurizer zone conditions and at increased chain speed. Three replications at various locations of the pasteurizer were conducted by placing the probe inside the cucumber to represent the cold spot. Process parameters were continuously recorded and product parameters critical to the process were measured and recorded as needed.
Results: The result demonstrated that the product samples consistently reached the lethal temperature of 160ºF with a minimum lethality of $F^{160}_{19.5}$ = 5.6. This satisfied the scheduled process requirement of 5-log reduction of pathogens in cucumbers with a pH<4.6. Solid-liquid ratio, Initial temperature, headspace, and container size were found to be critical factors.
Significance: Though the process applied in the industry is much severe than the minimum required for safety, optimizing this process based on the system in-use will help in improving product quality. This will also support the FDA filing for acidified foods. It is critical to consider the worst-case situation of the product, process and package and each of the manufacturers should validate their system.

P2-75 Validation of a Kimchi Recipe for Home Food Preservers

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Introduction: Kimchi has gained popularity among consumers for its potential health benefits and versatility. However, no recipes for home food preservers have been validated for safety and quality in the USA.

***Purpose*:** Determine critical food safety factors such as salt concentration and pH levels to decrease *Escherichia coli* K12 and *Staphylococcus aureus* populations during fermentation.

***Methods*:** Kimchi was prepared according to a traditional recipe, and salt concentrations were tested at 2.5% and 3% (w/v). Kimchi was inoculated with challenge organisms (*E. coli* K12 and *S. aureus*) and a control was established for each salt concentration, yielding three treatment conditions per replicate, for a total of three replicates. Control batches for each salt concentration were monitored for lactic acid bacteria (LAB). Organisms' survivability, LAB growth, and pH were monitored on Days 0, 2, 5, 7, and 14. Samples were stored at 22±1°C for Days 0 and 2, and at 4±0.5°C thereafter. Data were collected in a completely randomized design and analyzed using a mixed-model ANOVA. Pairwise comparisons were made at alpha = 0.05.

***Results*:** *E. coli* and *S. aureus* reductions were significantly affected by the interaction of sampling day and salt concentration. A greater reduction (1.5±0.11 log CFU/g) in *E. coli* population was observed on Day 5 when salt concentration was 2.5%, compared to 3% (0.8±0.13 log CFU/g; $p<0.05$). Similar trends were observed for *S. aureus* with reductions of 2.2±0.03 log CFU/g at 2.5% salt compared to 1.9±0.03 log CFU/g at 3% salt during the same period. LAB counts at 2.5% salt on Day 5 were 8.91±0.11 log CFU/g while 4.97±0.09 log CFU/g at 3% salt on the same period. pH decreased from Day 0 to 14 for all treatments ($p<0.05$), with the lowest reaching 4.2±0.16 for LAB.

Significance: Ensuring the microbial safety and validation of recipes is essential, enabling consumers to engage in safe home food fermentation while minimizing the potential for foodborne illnesses.

P2-76 Comparison of Acid Resistance of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. Strains in Brain Heart Infusion Broth

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Introduction: Challenge studies are conducted for acidified foods to demonstrate the safety and stability of the products and to follow FDA regulations (21 CFR 114). When designing challenge tests, it is important to include microorganisms that are resistant to acidity to represent the worst-case scenarios.

***Purpose*:** The objective of this study was to develop a simple method to consistently compare the resistance to acidity between microorganisms and within a genus at varied pH levels.

***Methods*:** Individual strains of *Escherichia coli O157:H7*, *Listeria monocytogenes* and *Salmonella* spp. were incubated in tryptic soy broth supplemented with yeast extract and 1% glucose for two consecutive days at 35°C to induce acid adaptation. Brain heart infusion (BHI) broth was acidified with 1N HCl to pH 3.0 – 4.0 and inoculated with pathogen cultures at 10^{6-7} CFU/mL. Inoculated BHI samples were incubated at 18°C and aliquots of samples were plated for residual population levels after different storage intervals using BAM methods. Three individual replicates were conducted.

***Results*:** Overall, acid resistance sequence of the selected pathogens, was *L. monocytogenes* > *E. coli* > *Salmonella*. For example, at pH 3.4, 72 h of incubation at 18°C resulted in < 0.5, 1.0 – 2.0, and 3.0 – 4.0 log reductions of *L. monocytogenes*, *E. coli*, and *Salmonella* spp. respectively. A similar trend was observed at other pH levels. Further, different strains of each genus exhibited differences in acid resistance. For example, *E. coli* 5-1-3 did not adapt to acidified environments at all tested pH levels, whereas *E. coli* 5-1-2 and 5-1-7 were relatively resistant and were only reduced by < 2.0 logs after 72 h of exposure at pH 3.4.

Significance: The study developed a method to investigate and compare the resistance of selected pathogen strains. The results could be used when selecting appropriate pathogen strains for challenge studies of acidified foods.

P2-77 Influence of Salt Concentration and Starter Culture on Survival of *Escherichia coli* O157:H7 during Sauerkraut Fermentation

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Introduction: Despite the rise in market trends of lacto-fermented foods, studies on process controls to validate pathogen growth control and establishing critical limits during sauerkraut production is limited.

Purpose: This project aims to investigate the effect of different process parameters (salt concentration and use of starter culture) on pathogen growth and fermentation conditions of sauerkraut over time.

Methods: Sauerkraut was prepared with 4mm-shredded cabbage pieces and mixed with sea salt for 15 minutes, then homogeneously inoculated with 10^8 CFU/g *Escherichia coli* O157:H7 cocktail (five strains). Samples were then filled in fermentation vessels with saltwater weight bags applied on top of cabbage to keep below brine level. Sauerkraut was made with different concentrations of salt and commercial starter culture (*Lactobacillus plantarum*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*). The pH, titratable acidity, lactic acid bacteria (LAB) and *E. coli* counts were monitored over fermentation at 22°C for 14 days. Measurements were made in triplicate (n=9) and analyzed using Two-Way ANOVA with the Tukey's *post hoc* test ($p<0.05$).

Results: Sauerkraut samples prepared with starter culture had relatively higher numbers of LAB counts by Day 2 (4 log increase) compared to naturally fermented ones. Samples with 1.6 and 3.2% salt with starter culture significantly lowered the pH below 4.6 by Day 3 (3.741 ± 0.044 and 4.097 ± 0.307 respectively), while the titratable acidity levels significantly increased by Day 2 (0.45% ± 0.10 and 0.31% ± 0.08 respectively) for these samples. All formulations resulted in a 5 log reduction of *E. coli* counts by Day 7, which sauerkraut consisting of 1.6% salt with starter culture had the fastest rate to reach below detection limit (<2 log CFU/g).

Significance: Identifying safety parameters for lacto-fermented sauerkraut that achieves optimal fermentation conditions to control pathogen growth can help provide technical support for food processors that need food safety plans that comply with the Food Safety Modernization Act (FSMA).

P2-78 Evaluating the Efficacy of Citric, Ascorbic, Malic and Tartaric Acids in a Model Acidified Food Formulation for the Reduction of *Escherichia coli* O157:H7

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Introduction: Trends in the food industry show consumers prefer minimally processed clean label foods; however, data supporting safe formulations for acidified foods (AF) with alternatives to acetic and benzoic acids are lacking.

Purpose: This study aimed to determine the efficacy of selected food acids for achieving a 5-log reduction (FLR) of *Escherichia coli* O157:H7 (STEC) in a model AF formulation.

Methods: A cocktail of five acid-resistant *E. coli* O157:H7 strains was prepared for inoculation (10^7 CFU/ml) of acid treatments in triplicate. Citric, malic, tartaric, and ascorbic acids were added to cucumber juice (CJ) and adjusted to pH 3.5 with HCl or NaOH as needed. Treatments were prepared based on selected protonated acid concentrations for citric (0.5 – 15mM), ascorbic (15 – 100 mM), malic (8.3 – 15 mM), tartaric (3.5 – 15mM), and control (acidified with HCl). CJ was used as a generic vegetable broth medium. Treatments were held at 10°C in an anaerobic chamber and sampled daily in a ten-fold dilution plating assay. Protonated acid concentrations were calculated using Matlab. Plates with no colonies were considered to have achieved a FLR.

Results: Citric acid delivered a FLR at pH 3.5 most effectively. A FLR was achieved with 5 mM or greater fully protonated citric acid by day two, while malic acid and tartaric acid (at 15 mM) had similar FLR times (four days) compared to the control treatments. Interestingly, ascorbic acid treatments at 100 mM did not achieve a FLR by the termination of the experiment, day 11. The standard deviation for measured FLR times were within +/- 1 day for all treatments.

Significance: These data will support manufacturers' use of acetic and benzoic acid alternatives when formulating AF. The protective effects of ascorbic acid should be considered when preparing AF, although mixed acid effects remain to be investigated.

P2-79 Evaluation of the BACT/ALERT 3D® for Rapid Detection of Spoilage Organisms in Alcoholic Juice and Water Beverages

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Background: The characteristics of alcoholic beverages allow the growth of a small class of organisms. Yeast can ferment in the acidic and low oxygen environment. Lactobacillus species, which are known for causing off-flavors in juices, have a high tolerance to alcohol and acidity. The BACT/ALERT 3D® (BTA) Microbial Detection System, used with BTA culture bottles, provides rapid detection of various microorganisms.

Purpose: A proof-of-concept study to show that the BTA methodology can detect spoilage organisms in 4 flavors of alcoholic beverages.

Method: BTA iLYM Culture Bottles were inoculated in triplicate with each beverage at 2 different volumes (1 mL and 5mL) to determine compatibility. Culture bottles were inoculated in triplicate with 1mL or 5mL of beverage followed by direct inoculation of organism suspension targeting 50cfu per test. *Candida albicans, Lactobacillus fermentum,* or *Lactobacillus paracasei* were used as targets. *Bacillus subtilis* was evaluated to identify the possible breakthrough of a non-spoilage (non-target) organism.

Results: One flavor resulted in false positives with 5ml sample size but all flavors were compatible at 1ml sample size. 100% of tests containing yeast were detected in <2 days in all test conditions. 100% of tests containing *Lactobacillus spp*. were detected in <2.5 days in all test conditions. Detection of *Lactobacillus spp*. was around 0.5 days slower in the 5mL sample volume than the 1ml sample volume. *Bacillus subtilis* (non-target organism) did not grow in any test condition. All BTA results were confirmed by traditional plating.

Significance: When spoilage organisms are present in low-alcoholic juice or water beverage at the levels tested during this study, the alternative method will detect these organisms in these product types without false positives from non-target organisms. The alternative method allows for faster spoilage detection and faster release of products compared with traditional methods.

P2-80 Microbial Safety of Cold Brewed Black Coffee during Retail

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Introduction: Cold brewed black coffee products, made with cool or ambient temperature water and potentially without thermal processing, may have pH and water activity favorable to pathogenic organism growth; thus, microbial safety at point-of-sale requires consideration of factors like storage time, temperature, and inherent antimicrobial properties.

Purpose: To evaluate the microbial food safety of concentrated and single strength cold brew coffee products, and to determine if temperature controls for safety should be required during retail.

Methods: Separate multi-strain cocktails of *Bacillus cereus*, proteolytic and non-proteolytic *Clostridium botulinum*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* were inoculated into cold brew concentrate (pH 5.28-5.39, 3.5-4.5% Brix) or single strength cold brew (pH 4.85-5.04, 1.6-2.0% Brix) at a target level of 2-3 log CFU/ml and were then stored at 29°C for 11 days. For each product, storage condition (aerobic or anaerobic), and sampling time (days 0, 1, 3, 5, 7, 11), 3-5 replicate samples were enumerated by plating. Additionally, *C. botulinum* samples were evaluated for the presence of botulinum toxin using the DIG-ELISA method. Two independent trials were performed.

Results: For both coffee products, population levels of *E. coli* O157:H7, *Salmonella*, and *S. aureus* declined below the detection limit (1.0 log CFU/ml) in ≤3 days. Population levels of *L. monocytogenes* declined more slowly in the concentrated cold brew under anaerobic storage conditions compared to aerobic storage conditions (p<0.05). Growth of *B. cereus* and *C. botulinum* was not observed over the storage period, and botulinum toxin was not detected.

Significance: The results of the challenge studies demonstrate that the cold brew black coffee products did not support growth and/or toxin production of pertinent pathogens identified for this product category. Therefore, temperature controls for safety should not be required for these cold brewed black coffee products, as formulated for this study.

P2-81 Improving Microbial Safety of Non-Heat Treated Energy Drink Using Novel Antimicrobials

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Introduction: Energy drinks have surged in popularity as a favored choice for consumers seeking a quick energy boost and enhanced mental alertness. Energy drinks, particularly non-heat-treated varieties, are susceptible to contamination by foodborne pathogens, posing significant health risks to consumers if not adequately controlled.

Purpose: This study aims to identify the effectiveness of novel natural antimicrobials, Prolong Liquid 2.0 (L) and sodium acid sulfate (SAS), to improve microbial safety of non-heat-treated energy drinks.

Methods: A baseline energy drink formulation was created to resemble common market offerings. Samples were individually inoculated with *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Escherichia coli* O157:H7 to achieve a final concentration of approximately 7.5 Log CFU/mL. The inoculated samples were then treated with SAS at concentrations of 0.1%, 0.5%, and 1.0%, L at concentrations of 0.1%, 0.5%, 1.0%, and 2.0%, or a combination of Sodium Benzoate (SB) at 0.1% and Potassium Sorbate (PS) at 0.1% each, and stored at ambient temperature. Samples were aseptically withdrawn at 0, 24, 48, and 72 hours and analyzed for the survival of the target pathogens using standard microbiological methods. All experiments were conducted in triplicate. Statistical analysis of the time-series data was performed using repeated measures one-way analysis of variance (RM-ANOVA) and the Holm-Sidak test for pairwise multiple comparisons to determine significant differences ($p < 0.05$) between treatments.

Results: The antimicrobials SAS 0.5%, 1.0%, and were the most effective among all treatments investigated. The antimicrobial treatments SAS 0.5%, and 1.0% reduced *L. monocytogenes*, and *S.* Typhimurium to non-detectable levels within 48, and 24h, respectively. For *E. coli* O157, only SAS 1.0% was able to reduce the pathogens to a non-detectable level within 24 h, while it took 48 h for L 2.0%, SB+PB to achieve the same reduction.

Significance: This study revealed that SAS 0.5%, 1.0%, and L 2.0% could become alternative antimicrobials for the energy drink industry.

P2-82 Rapid Detection of Microbial Contamination in Different Low Acid Beverages using Neogen Microbial Luminescence System (MLSII)

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Introduction: Beverages processed with ultra-high temperature treatments (UHT) or extended shelf life (ESL) have an increased demand in the food market. Microbial analysis for testing commercial sterility is traditionally done with agar plating after product enrichment, which can take several weeks to confirm a negative result delaying product release.

Purpose: To evaluate a microbial ATP-bioluminescence-based method as a rapid test for screening commercial sterility in five different types of low acid beverages at different enrichment times and compared to agar traditional method.

Method: Five types of low acid beverages (dairy protein, protein-based water, plant-based protein, cold brew coffee and non-dairy beverages) were inoculated with low (<10 CFU/container) and high levels (>100 CFU/container) utilizing different types of microorganisms (4) per beverage, including *Bacillus circulans*, *Pseudomona aeruginosa*, *Bacillus cereus*, *Geobacillus stearothermophilus* and *Saccharomyces cerevisiae*. A negative control (unincoculated) was also included for each matrix. Inoculated containers were enriched at 32°C, 55°C or 25°C, for 1, 2, 3, 5 and 7 days. After enrichment beverages were screened for commercial sterility utilizing standard methods agar (SMA) as a reference method and a Neogen MLSII based on the detection of microbial ATP.

Results: The study demonstrated that ATP-bioluminescence-based alternative method can detect the presence of microbial contamination >48 hours sooner than SMA to the beverages included in this evaluation. Results showed 100% agreement between the alternative and reference methods. The organisms that grew in the beverages were detected as soon as 48 hours at the low levels and 24 hours at the high levels. The microorganisms used in this study were unable to survive in the protein-based water and the cold brew beverages, confirmed by no detection on agar plates.

Significance: Microbial ATP bioluminescence can provide a rapid result to screen commercial sterility by significantly reducing time to product release and thus reducing inventory hold times.

P2-83 16S rRNA Nanopore Sequencing for Non-targeted Wastewater-based Environmental Analysis of Foodborne Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne outbreaks are a significant public health issue globally. As interest in wastewater-based epidemiology increases, 16S rRNA nanopore sequencing can be used for environmental assessments of foodborne pathogens.

Purpose: The aim of this study was to detect and quantify species of foodborne pathogens in wastewater using 16S rRNA nanopore sequencing.

Methods: Total DNA was extracted using the AllPrep PowerViral DNA/RNA Kit from 26 wastewater samples obtained from the Guelph wastewater treatment plant (Guelph, Ontario, Canada). All the samples were analyzed by RT- qPCR using SureTect Assays (ThermoFisher) for *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes* and *Campylobacter* spp. Full-length 16S rRNA genes were amplified by the 16S Barcoding Kit (SQK-16S024, Oxford Nanopore Technologies, Oxford, UK). The prepared DNA library was loaded onto the R9.4 flow cell (FLO-MIN106) and sequenced on the GridIon Mk1™. MINKNOW software ver. 1.11.5 (Oxford Nanopore Technologies) was used for data acquisition. Sequences were quality filtered, trimmed, and analyzed using standard bioinformatics tools.

Results: RT-qPCR results indicated the presence of *stx*-positive and *eae*-positive *E. coli* isolates, and *Salmonella* spp. in a majority of the wastewater samples. Of the 4,283,655 sequences that were obtained from 16S rRNA gene amplicon sequencing, 99.999% were successfully classified. The majority of bacterial species with ≥1% relative abundance and constituting the top 13 were either known or emerging foodborne pathogens. The emerging foodborne pathogen *Aliarcobacter cryaerophilus* (19%) was the most abundant bacteria. In addition, five different *Arcobacter* species (Σ = 31.4%), and *Acinetobacter baumannii* (2.64%) were detected. Of note, *E. coli* and *Salmonella enterica* had relative abundances of 1.1% and 0.35%, respectively.

Significance: The present study demonstrates the analytical advantage of sequencing the full-length 16S rRNA gene for non-targeted analysis of foodborne pathogens during wastewater-based surveillance. The method is particularly useful for identification of emerging foodborne pathogens and can supplement existing food and animal-based surveillance approaches.

P2-84 *Salmonella enterica* Serovar Braenderup: Comparative Genomic Analysis of Global Clinical and Non-Clinical Isolates to Reveal Population Structure, Source Attribution Trends, and Putative Clusters

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❖ ***Developing Scientist Entrant***

Introduction: The foodborne pathogen *Salmonella enterica* subsp*. enterica* serovar Braenderup (*S.* Braenderup) is a clinically and epidemiologically significant serovar with a higher prevalence in the Southeastern United States. It is the 5th most common clinical *Salmonella* serovar, causing 5% of confirmed *Salmonella* outbreaks.

Purpose: This study applies whole-genome sequencing (WGS) and bioinformatics approaches to help elucidate *S.* Braenderup population structure, source attribution trends, and identify potential outbreak clusters.

Methods: Sequenced clinical *S.* Braenderup BioSample IDs were obtained (n=799) from state public health laboratories (Tennessee, Kentucky, Virginia, South Carolina, Georgia, Alabama, Arkansas, and Louisiana) as part of routine surveillance. Raw reads from NCBI SRA were trimmed and assembled into contigs. Global clinical (n=5,153) and non-clinical (n=1,053) assemblies were downloaded from the NCBI. Assemblies that did not meet the inclusion criteria (≤150 contigs, length 4-5.1 Mbp, 51.9-52.6% GC, and >10x coverage) were excluded. The final dataset (n=6,436) was used to determine overall phylogeny based on core SNPs. The population was subdivided using FastBAPS. ClustFinder was used to identify potential outbreak clusters (≤10 hqSNP threshold).

Results: The phylogeny consisted of clade I (n=3,593; 85% clinical isolates), clade II (n=2,261; 86% clinical isolates) and clade group III (n=682; 82% clinical isolates). The top three sources of non-clinical isolates in clade I (n= 536; 15%) were produce (29%), poultry (22%), and environment (water/soil/sediment) (19%). The top two sources of non-clinical isolates in clade II (n=326; 14%) were poultry (56%) and environment (water/soil/sediment) (19%). We identified potential outbreak clusters in clade I (n=102), clade II (n=53), and clade group III (n=18).

Significance: This study provides insight on the relationship between clinical and non-clinical strains and may provide a foundation for future work to investigate the transmission of *Salmonella* Braenderup from contaminated food and the environment to humans.

P2-85 Estimating the Burden of Foodborne Illness for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan, 2006–2021

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Introduction: In Japan, the numbers of food poisoning incidence and cases are reported mandatory; however, these do not exactly reflect the real burden of foodborne illnesses due to the passive surveillance nature. We have been estimating the real burden of foodborne diseases for *Campylobacter*, *Salmonella* and *Vibrio parahaemolyticus* in Japan since 2006.

Purpose: Estimating the burden of foodborne illnesses associated with three pathogens in Japan from laboratory confirmed numbers of infections.

Methods: Data on laboratory-confirmed infections of three pathogens were collected from clinical laboratories that test stool samples submitted from all over Japan or from Miyagi Prefecture, from January 2006 to December 2021. The physician consultation rate and the stool submission rate were estimated from telephone population surveys conducted for whole of Japan and for Miyagi prefecture. We merged the telephone survey data conducted in 2016 with previous data. Each estimate was introduced into the Monte-Carlo simulation model as a probability distribution, which was run for 10,000 iterations.

Results: The estimated mean numbers per year of foodborne illnesses for *Campylobacter*, *Salmonella* and *V. parahaemolyticus* in whole of Japan were 3.8 -13.6 million, 0.9 -2.8 million, and 2-438 thousand during 2006-2021, respectively. Those estimated for whole of Japan from data on Miyagi prefecture were 0.64 -1.6 million, 78-190 thousand, and 0-63 thousand during 2006-2021, respectively. The numbers of reported foodborne illnesses per year in Japan during 2006-2021, for *Campylobacter*, *Salmonella* and *V. parahaemolyticus*, were 760 -3,200, 320-3,600 and 3-1,300, respectively.

Significance: These data reveal a significant difference in numbers and trends between our estimates of burden of foodborne illnesses and the reported foodborne disease cases associated with three pathogens. Need for continuing active surveillance system to complement the present passive surveillance is strongly suggested, in order to identify and prioritize food safety measures more precisely and to monitor the effectiveness of risk management options.

P2-86 Diarrheagenic *Escherichia coli* and *Salmonella* spp. Contamination of Food and Water Consumed by Children with Diarrhoea in Maputo, Mozambique

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Introduction: Foodborne illnesses are a public health concern worldwide, and mainly in high-income countries, where one child in 13 dies before their fifth birthday. In Mozambique, recent data indicate a high disease burden of diarrhea and about 500,000 cases were caused by foodborne pathogens in children under five years old.

Purpose: Therefore, this study was conducted to determine the Enterobacteriaceae contamination of food and water in urban (Kamaxakeni) and rural (Marracuene) areas of Maputo consumed by children under five years of age with diarrhea.

Methods: A semi-structured questionnaire was used to collect data on the demographic characteristics and the food consumed a week before the children experienced their diarrhea episodes. Food and water samples collected from these households were delivered to the hygiene and food technology laboratory and analyzed for the presence of diarrheagenic pathogens. The data analysis was based on a binomial regression model to ascertain the factors associated with children's food contamination.

Results: The prevalence of Diarrheagenic *E. coli* (DEC) and *Salmonella* spp. was 8.6%. DEC was most prevalent in cereal foods and water (6.0%), while *Salmonella* spp. was mainly detected in cereal foods (0.8%). *Salmonella* spp. was only detected in water from the urban area. The presence and type of diarrheagenic pathogens were associated with demographic characteristics, marital status, and the food consumed by children under two years old (infant formula, fruit puree, ready-to-eat meals and bottled water).

Significance: From this study, it can be concluded that the prevalence of food pathogens in food and water consumed by children under five of age living in urban and rural districts of Maputo differ, being higher in rural area (4.6%). This study shows that the type of infant foods consumed in Maputo districts and the demographic characteristics of the caretakers contribute to diarrhea in children under five years.

P2-87 Antimicrobial-Resistant Strains of Major Nontyphoidal *Salmonella* Serotypes Isolated from Humans and Retail Chicken in the United States – National Antimicrobial Resistance Monitoring System, 2011–2020

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Introduction: *Salmonella* is transmitted commonly through food, causing ~1.3 million illnesses in the United States annually. Chicken is an important source of antimicrobial-resistant *Salmonella* strains.

Purpose: We described antimicrobial resistance among major *Salmonella* serotypes isolated from humans and retail chicken during 2011–2020.

Methods: We included antimicrobial susceptibility testing (AST) and whole genome sequencing data among *Salmonella* isolates from humans and retail chicken submitted to the National Antimicrobial Resistance Monitoring System (NARMS). We examined clinically important resistance, including ceftriaxone resistance and decreased susceptibility to ciprofloxacin (DSC), determined by AST among major serotypes. Multidrug-resistant Infantis (REPJFX01) from humans was defined by PulseNet as isolates related within 0–82 alleles by core genome multilocus sequence typing, typically harboring *gyrA87* mutation conferring DSC and pESI plasmid carrying multiple resistance genes (e.g., $bla_{\text{CTX-M-65}}$ conferring ceftriaxone resistance). We included phylogenetically related Infantis sequences in the National Center for Biotechnology Information that were submitted by NARMS and PulseNet to identify chicken isolates related to REPJFX01. We used logistic regression to compare average resistance percentages for 2016–2020 vs. 2011–2015.

Results: During 2011–2020, the four most common serotypes among 3,458 chicken isolates were Kentucky (34.6%), Infantis (17.2%), Typhimurium (16.7%), and Enteritidis (15.2%). Enteritidis, Typhimurium, and Infantis accounted for 33% of 23,343 human isolates; Kentucky accounted for 0.3%. There were increases (p<0.05) in DSC among Enteritidis from humans (23% [2016–2020], 9% [2011–2015]) and chicken (14%, 0%), and Infantis from humans (25%, 3%) and chicken (83%, 5%); ceftriaxone resistance among Infantis from humans (16%, 4%) and chicken (42%, 10%); and REPJFX01 from humans (18%, 1%) and chicken (83%, 5%). Increases among Typhimurium were not noted.

Significance: Enteritidis and Infantis from humans and chicken showed increases in resistance. Infantis related to REPJFX01 from humans is now the predominant strain, causing increased DSC and ceftriaxone resistance, among Infantis from NARMS retail chicken. These findings can inform priorities for reducing contamination of chicken and human illnesses caused by *Salmonella* serotypes.

P2-88 Combined with Metabolomic and Adverse Outcome Pathway Analysis to Assess the Genotoxic Effects and Molecular Mechanisms Induced by the Food Contaminant Glycidol

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Introduction: Glycidol, a contaminant arising from the deodorization process in vegetable oil refining, has been reported to induce toxicity to various organs. Classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC) due to its multiple tumor-promoting effects. Glycidol's alterations in carcinogenicity, metabolism pathways, and molecular toxicity mechanisms remain unclear.

Purpose: This study aims to investigate the adverse outcome pathway of genotoxicity mechanisms induced by Glycidol, along with exploring alterations in metabolites associated with its toxicity.

Methods: Sub-acute toxicity of Glycidol was studied in C57BL/6 mice through oral administration. Biochemical values for liver and renal function, as well as observation of histopathological damage in kidneys and other organs was conducted. Metabolomic analysis focused on urine metabolites. Mechanistic studies involved treating NRK-52E cells with Glycidol, utilizing fluorescent dyes, comet assays, transmission electron microscopy, flow cytometry, and western blotting to investigate DNA damage, cell cycle arrest, mitochondrial dysfunction, and programmed cell death induced by Glycidol.

Results: The sub-acute toxicity study revealed kidney injury induced by Glycidol, evident in histopathology. Interestingly, this damage partially recovered after a 28-day cessation of Glycidol exposure. Metabolomic analysis identified Glycidol-induced alterations in pathways related to DNA damage and mitochondria. In vitro studies demonstrated Glycidol's ability to induce micronuclei formation, increase tail length, percentage, and tail moment in the comet assay. Elevated levels of DNA damage-related proteins γ-H2AX and p-ATM were observed, along with the inhibition of CDK1/cyclin B and G2/M phase cell cycle arrest. Additionally, Glycidol up-regulated Drp1 and down-regulated Mfn1 expression, increasing ROS production, decreasing mitochondrial membrane potential, resulting in mitochondrial dysfunction, mitophagy, and pyroptosis.

Significance: This study provides valuable insights into how Glycidol influences metabolism and induces genotoxicity both in vitro and in vivo. These identified mechanisms may serve as potential preventive targets to mitigate Glycidol-induced toxicity.

P2-89 Occurrence and Risk Assessment of Domoic Acid and Its Isomers in Seafood Marketed in South Korea

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Introduction: Domoic acid (DA), co-contaminated with isomers, is responsible for incidents of amnesic shellfish poisoning. In Korea, there is an increasing importance in safety management of seafood, however, the study related to the occurrence of DA and isomers is insufficient.

Purpose: This study presented the occurrence of DA and isomers in seafood marketed in Korea and a risk assessment was performed for DA and epi-DA.

Methods: Total 347 seafood samples including 19 species of crustacea, bivalve, fish and gastropoda were analyzed by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) for the simultaneous determination of DA and its isomers, and ultraviolet detection (HPLC-UV) to determine DA. The cleanup with solid phase extraction was used to minimize the false positive results from the matrix interference.

Results: DA and its isomers were detected in 15 samples (*Crassostrea gigas*, *Engraulis japonicus*, *Fulvia mutica*, *Mactra quadrangularis*, *Mizuhopecten yessoensis*, *Mytilus coruscus*, *Mytilus galloproinacialis*, *Scapharca broughtonii*). In *Engraulis japonicus*, DA concentrations of 358.8 μg/kg and 99.2 μg/kg were detected by HPLC-MS/MS and HPLC-UV, respectively, but epi-DA was not detected in all samples. The acute dietary exposure values of the consumer group by HPLC-UV results were 0.0606-0.0800 μg/kg bw/day and those with HPLC-MS/MS results were 0.2171-0.2865 μg/kg bw/day. They were lower than the acute reference dose of 30 μg/kg bw evaluated by the European Food Safety Authority. Therefore, the results of this study indicate that the exposure to DA and epi-DA in seafood marketed in Korea is unlikely to pose a health risk to consumers.

Significance: In this study, a method for the determination of DA and its isomers by HPLC-UV and HPLC-MS/MS with SPE cleanup was successfully validated and applied to 347 seafood samples. This is the first study of quantifying DA isomers and performing risk assessment with DA and epi-DA in seafood marketed in Korea.

P2-90 Natural Occurrence of Microcystins and Nodularin in Agricultural Products Marketed in South Korea by Liquid Chromatography-Tandem Mass Spectrometry

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Introduction: Microcystins (MCs) and nodularin (NOD), known as hepatotoxin, are peptide toxins that naturally occurred by cyanobacteria blooms. Due to tropic and global climate changes, cyanobacteria blooms have been increased, MCs and NOD occurrences also have been increased. MCs and NOD mainly occurred in freshwater; however, agricultural products can be contaminated in form of bioaccumulation if they are irrigated by water contaminated with MCs and NOD. But the determination of MCs and NOD occurrence in agricultural products marked in South Korea has not been performed.

Purpose: In this study, natural occurrence of 7 cyanobacterial peptide toxins (CPTs; MC-LR, -YR, -RR, -LA, -LF, -LY and NOD) were determined in agricultural products marketed in South Korea by using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: The 120 collected samples were classified into 6 categories (cereal grains, vegetables, fruits, oilseeds, spices, and algae). For the sample preparation, solid phase extraction was conducted for cleanups and the determination of 7 CPTs was carried out by LC-MS/MS. The performance of the method was measured in rice, grape and lettuce and evaluated in accordance with the Association of Official Analytical Chemists (AOAC) and Codex guidelines.

Results: Good linearity of the calibration curves was obtained for all CPTs in all validated matrices (R^2>0.99) with LODs ranged from 0.057-0.308 μg/kg and LOQs ranged from 0.148-0.791 μg/kg. Recovery and precision were 70.65-110.7% and 0.89-10.91%, respectively, and satisfied the requirements of AOAC and Codex guidelines. In most of the 120 samples, the concentrations of 7 CPTs were below 1 μg/kg, the guideline value of drinking-water by World Health Organization.

Significance: To the best of our knowledge, this is the first research to determine MCs and NOD in agricultural products marketed in South Korea. Furthermore, the result may serve as a scientific basis for risk assessment of the CPTs in agricultural products.

P2-91 Exploring Genotoxicity and Carcinogenicity Effects of ZnO Nanoparticles: Insights from in-Vitro Studies on NH2-ZnO and COOH-ZnO Coated ZnO Nanoparticles

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❖ ***Developing Scientist Entrant***

Introduction: Zinc oxide (ZnO) nanoparticles (NPs), commonly used in the food industry for their antibacterial and coloring properties, may elicit unique toxic responses due to their altered properties resulting from reduced size compared to their original form.

Purpose: The objective of this study was to explore the possible genotoxicity and carcinogenicity effects of ZnO NPs.

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Methods: NH_2-ZnO and COOH-ZnO coated ZnO NPs were employed in this study to assess their impact. The focus was on locating and quantifying nanoparticle entry into cells. Genotoxicity and carcinogenicity were evaluated using IF Staining (γ-H2AX), the In Vitro Mammalian Cell Micronucleus Test (OECD TG 487), and the In Vitro BHAS 42 cell transformation assay (OECD TG). Additionally, the study explored KEs of AOPs triggered by ZnO NPs, with NGS used to probe potential RNA regulation.

Results: The results of the micronucleus test showed that NH_2-ZnO and COOH-ZnO NPs increased in the proportion of micronuclei in Bi-nucleated CHO-K1 cells in a dose-dependent manner, suggesting genotoxicity. Moreover, γ-H2AX IF staining revealed DNA double-strand breaks. The potential KEs associated with genotoxicity induced by ZnO NPs may be attributed to an increase in reactive oxygen species (ROS) production and the occurrence of oxidative lesions in DNA. Western blotting results of DNA damage-related proteins p-ATM/total ATM increase in CHO-K1 cell after expose to ZnO nanoparticles. In Vitro BHAS 42 cell transformation assay results showed that NH_2-ZnO and COOH-ZnO exhibited a higher transformation ratio in the initiation and promotion phase compared to the negative control group. Based on the results, we conducted NGS to identify the potential RNA molecules that ZnO NPs may regulate.

Significance: Our study highlights potential genotoxicity and carcinogenicity effects of ZnO nanoparticles. Further safety evaluations are necessary for the widespread future application of nanoparticles in food.

P2-92 Distillation as an Alternative Use for Deoxynivalenol-Contaminated Wheat or Rye: Minimal Carryover of Deoxynivalenol into Distilled Spirits

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❖ ***Developing Scientist Entrant***

Introduction: Managing deoxynivalenol (DON) risks is crucial for the sustainability of North-Central Region small grain farms. One approach involves profitable utilization of contaminated grain resources, addressing potential losses from food safety concerns.

Purpose: This study explored distillation as a high-value alternative for utilizing DON-contaminated grain.

Methods: Naturally DON-contaminated rye and wheat were used in two pilot-scale distillation runs involving milling, mashing, fermentation, and distillation. The ground grain, porridge, fermented mash, and post-distillation mash were sampled during process. For the distilled spirit, 29 fractionated samples, each containing 125 ml, were collected starting with the first drop of liquor. The fractionated samples were sequentially combined into 6 pooled samples of up to 5 individual fractions. If a pooled sample had a DON level above the limit of quantification, samples of the pool were tested individually. All samples were tested by ELISA with a limit of quantification at 0.05 ppm and a limit of detection at 0.01 ppm.

Results: For both rye and wheat runs, DON levels in all distillate fractions were consistently below the FDA advisory level (1 ppm), reducing from barely quantifiable to below 0.01 ppm. The DON levels in ground rye and wheat were 3.62 and 2.69 ppm, respectively. In the rye distilled spirit, the first pooled sample had a DON level of 0.1 ppm, and the first two fractions of that pool had DON levels of 0.1 and 0.06 ppm. In the wheat distilled spirit, the first pooled sample had a DON level of 0.05 ppm, and the first fraction of that pool had DON level of 0.12 ppm. All other distilled spirits had DON levels below 0.01 ppm.

Significance: The results showed that distilled liquor from DON-contaminated rye and wheat contains very low DON levels at most. From a food safety perspective, considering DON-contaminated grain as an ingredient for distilled spirits appears viable.

P2-93 Leveraging Diffusion and Fine-Tuned Large Language Models for Mycotoxigenic Fungi Prevention and Mitigation: A Focus on Predicting Protein-Protein Interactions between Maize and Fusarium

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Introduction: *Fusarium graminearum* is a plant pathogenic fungus that produces several toxic metabolites (i.e. deoxynivalenol, fumonisins) and can infect the edible portion of susceptible cereal crops (maize, wheat, etc.) at many stages of the supply chain. Due to the critical role of proteins in driving both fungal infection (e.g. effectors) and the plant immune response, *in silico* methods of predicting protein-protein interactions (PPIs) in the host-pathogen crosstalk have been heavily studied. However, machine learning-based methods for predicting plant host-pathogen interspecies PPIs are hindered by the limited experimentally validated data in current databases.

Purpose: The objective of this study was to overcome this obstacle through recent advances in artificial intelligence and predict interspecies PPIs between *F. graminearum* and maize (corn; *Zea mays* L.).

Methods: Candidate fungal effectors from *F. graminearum* were first identified using computational biology tools (EffectorP; SecretSanta). The binding residues of each effector were then imputed using ESMBind - a fine-tuned protein language model. With these residues, the structure of predicted binders to each effector were then generated using RFdiffusion. FoldSeek was then used to identify maize proteins which shared structural similarity to the binders (and thus may bind to the effector). The protein-protein interface of each presumptive PPI was validated using ColabFold.

Results: A total of 606 PPIs were identified for 36/41 of the effectors. The involvement of the interacting maize proteins in various aspects of membrane trafficking (i.e., Snf7, p=1.22E-26; SNARE, p= 0.000131) suggests that the fungus may be 'hijacking' the plant immune response through structural similarity to produce extracellular metabolites.

Significance: Although the PPIs need to be experimentally validated, this study presents a framework for generating predicted PPIs for virtually any plant or pathogen of interest. The results of this study will be accessible through publicly available databases (MaizeGDB-*https://www.maizegdb.org/*; GrainGenes-*https://wheat.pw.usda.gov/*) and may be used for developing bio-based interventions for mycotoxin control in staple food crops.

P2-94 Epigenotoxicity of Food-Grade Titanium Dioxide in Human Cell Lines

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Introduction: Food-grade titanium dioxide (TiO2) is widely used the food industry. Significant toxicity of food-grade TiO2 has, however, been recognized, suggesting considerable risk to human health. To evaluate fully its toxicity, assessment of the epigenetic action of this nanomaterial is critical. However, only few studies are available examining the capability of food-grade TiO2 to alter epigenetic integrity.

Purpose: In the present study, the effects of food-grade TiO_2 exposure on histone modification and histone modifying enzymes expression, a major epigenetic mechanism, were investigated in human colorectal (Caco-2) and liver (HepG2) cell lines.

Methods: Expression levels of histone modifying enzymes were determined by array analysis using the Human Epigenetic Chromatin Modification Enzymes RT² Profiler™ PCR Array. qRT-PCR analysis confirmed the array results. Histone H3 and H4 modifications were assessed by array analysis using the EpiQuick™ Histone H3 or H4 Modification Multiplex Assay. Verification was done by Western blot analysis.

Results: Fourteen genes with altered expression were identified after exposure to food-grade TiO_2 (Caco-2 cells: *PRMT5, PRMT8, PAK1, HDAC10, KDM4C, KMT2A*; HepG2 cells: *AURKC, HDAC10, KAT2A, KDM4A, KDM4C, SETD7, SUV39H1, USP21*). Thirteen histone modifications were identified with altered levels (Caco-2 cells: H3K9me3, H3K27me2, H3K36me3, H3K9ac, H3K18ac, H4K8ac, H4K20m2; HepG2 cells: H3K9me2, H3K27me3, H3K79me1, H3K9ac, H3K18ac, H4K20m2). Alteration in the levels of these histone modifying enzymes and histone modifications is associated with diseases.

Significance: The findings from this study clearly demonstrate the impact of food-grade TiO2 exposure on histone modifying enzymes expression and histone modification in human cell lines, supporting potential involvement of this epigenetic mechanism in the toxicity of the nanomaterial. Hence, epigen-

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etic studies are critical for complete assessment of potential risk from food-grade TiO2 exposure.

P2-95 Toxicological and Microbiological Impact of Microplastics and Nanoplastics in Food Safety

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Introduction: The ubiquity of microplastics and nanoplastics in the environment presents a burgeoning challenge to food safety. Latest findings in Europe and USA have shown incredible concentrations of microplastics and nanoplastics in foods and bottled water. But how big is the risk for food safety and human health is not clear.

Purpose: Part of Horizon2020 project "ImpTox" seeks to unpack the complex interactions between these minute pollutants and the safety of our food supply, with a special emphasis on their impact on cellular health, and their interaction with foodborne pathogens and environmental microbiome.

Methods and Results: The toxicological focus delves into the potential health risks these particles pose, including their role in the bioaccumulation of harmful cyanotoxins, and direct toxic effects on human cells in function of dose an exposure time. Our findings showed that the impact of microplastics and nanoplastics on cellular metabolism and mitochondrial function depends on the type, size and concertation of nanoplastics. This has been noticed by using live cell imaging (Sartorius Incucyte SX5) and Agilent Seahorse XF Analyzer interrogating mitochondrial respiration and glycolysis. Moreover, we have tested impact of nanopalstics on boar semen motility as an ex-vivo system for toxicological assessment showing that ATP-dependent motility can be hampered by presence of nanoplastics such as PS, PP and PET. In parallel, our results showed important microbiological implications, especially regarding the facilitation of biofilm formation and virulence expression by foodborne pathogens like *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes*, and *Campylobacter jejuni*. Furthermore, the ability of microplastics to act a carrier of marine microbiota in function of environmental conditions (temperature, salinity etc) has been proven, including presence of antibiotic resistance genes.

Significance: This presentation aims to provide a view on current understanding of risks related to microplastics and nanoplastics in food.

P2-96 Multi-Mycotoxin Profiling in Traditional Korean Fermented Soybean Paste (*Doenjang*) and Its Raw Material (*Meju*) Using Liquid Chromatography Tandem-Mass Spectrometry with Immunoaffinity Cleanup

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Introduction: *Doenjang*, a traditional Korean fermented soybean paste, and *Meju*, a key raw ingredient in its production, are susceptible to contamination by mycotoxins during spontaneous fermentation, posing potential health risks.

Purpose: This study aimed to (ⅰ) develop a simultaneous determination method for twenty mycotoxins in *Meju* and *Doenjang* matrices and (ⅱ) investigate the diverse mycotoxin profiles in these fermented products.

Methods: Determination of 20 mycotoxins in *Meju* (n = 131) and *Doenjang* (n = 190) was performed by UPLC-QqQ-MS/MS using multifunctional immunoaffinity cleanup. The mycotoxins analyzed were aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A, (OTA), zearalenone (ZEN) and metabolites of ZEN (zearalanone, α-zearalenol, α-zearalanol, β-zearalenol, β -zearalanol), and fumonisin Bs (FB1, FB2, FB3).

Results: The developed method showed good performance in terms of linearity (R^2>0.999), limit of quantitation (<8.49 μg/kg), recoveries (86.6−109.7%), and repeatability (<15.3%). Among 20 mycotoxins analyzed, AFB1 was found to be the most prevalent mycotoxin in both *Meju* (31%) and *Doenjang* (23%), with concentrations ranging from 0.05−611.96 μg/kg and 0.11−79.10 μg/kg, respectively. Of a total of 321 samples tested, 37% of *Meju* and 75% of *Doenjang* were co-contaminated with two or more mycotoxins. The most frequent paired combinations were AFB1+AFB2 (22%), AFB1+AFG1 (17%), and AFB2+AFG1 (15%) in Meju, and OTA+ZEN (38%), FB1+FB2 (35%), and OTA+FB1 (33%). Positive correlations were observed between the contamination levels of AFB1 and AFB2, AFB1 and ZEN, AFB1 and FB1, and ZEN and FB1.

Significance: Our findings highlight AFB1 as a major contaminant in both Meju and Doenjang. Further research is needed for implementing control measures to mitigate aflatoxin-related public health risks in these fermented products.

P2-97 Comparative High-Throughput Sequencing Analysis of Fungal Communities in Aflatoxin-Contaminated and Non-Contaminated *Meju*, the Starter for Naturally Fermented Korean Soybean Paste

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Introduction: Spontaneously fermented soybean paste is vulnerable (susceptible) to contamination by aflatoxigenic fungi, leading to the production of aflatoxins. *Meju*, a fermented soybean brick, plays a pivotal role as a starter in the production of naturally fermented soybean paste, known as *Doenjang* in Korea.

Purpose: This study aims to (ⅰ) characterize the fungal diversity in relation to aflatoxin contamination and (ⅱ) identify fungi potentially associated with aflatoxin production in *Meju* and *Doenjang*.

Methods: Determination of AFs was carried out by high-performance liquid chromatography coupled with fluorescence detection. Genomic DNA was extracted using the DNeasy PowerSoil Kit (Qiagen), and internal transcribed spacer two regions were sequenced using a MiSeq Reagent Kit v2 on an Illumina Miseq platform. Taxonomic assignment was performed using the UNITE database.

Results: For taxonomic classification, *Aspergillus flavus* and *Mucor racemosus* were the predominant species in *Meju* and *Doenjang*, respectively. α-Diversity metrics, including Chao1, ACE, observed OTUs, and phylogenetic diversity, were significantly higher in aflatoxin-free *Meju* and *Doenjang* samples (p<0.05). β-Diversity analysis via principal coordinate analysis revealed varying degrees of clustering in *Meju* samples based on aflatoxin contamination. Linear discriminant analysis effect size identified 38 and 7 discriminative taxa (phylum to genus) in *Meju* and *Doenjang* samples, respectively. All taxa, except *Ascomycota*, were significantly abundant in both aflatoxin-free *Meju* and *Doenjang* samples (p<0.05). At the species level, all significant taxonomic biomarkers were abundant in aflatoxin-free *Meju* and *Doenjang* samples, except for *A. subolivaceus*, *A. terricola*, and *A. parasiticus*.

Significance: The findings provide valuable insights into the intricate relationship between fungal communities and aflatoxin contamination in *Meju*, suggesting a potential biological control strategy for mitigating aflatoxin contamination.

P2-98 Mitigating Acrylamide: Efficacy of Asparaginases and Green Tea Extract in Enhancing Food Safety in Breakfast Cereals

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❖ ***Developing Scientist Entrant***

Introduction: In heated food, the Maillard reaction between asparagine and a carbonyl source can result in the formation of acrylamide (AA), which is classified as "probably carcinogenic to humans" (1). Due to high levels of free asparagine (precursor) in cereals, AA remains a food safety concern.

Purpose: This study aims to investigate the effect of asparaginases and green tea extract in different concentrations for the reduction of AA in breakfast cereals while maintaining positive textural and color properties.

Methods: Acrylamide ELISA kit was used for AA analysis, a three-point bend rig was used to snap cookies for texture analysis and C-Cell and Color Muse were used and compared for color analysis. A total of 84 samples were analyzed for this project.

Results: We found that heat-stable asparaginases are more efficient in reducing AA. A reduction of up to 928 μg/kg in oat flakes and up to 360 μg/kg in wheat flakes was found when using these asparaginases. On the other hand, the addition of green tea extract showed no reduction in most samples

tested. Increasing the concentration of asparaginases did not play a significant role, nor did the incubation temperature. Lower asparagine grain formed less AA in the final product. Texture and color were found to have some variation within one batch due to the higher weight loss for flakes on the surface of the batch, however, no significant changes were noticed. The outcomes support the advantages of using asparaginases because the process conditions remain the same and the product has the same textural properties and no color change after the treatments.

Significance: This research highlights its significance in maintaining AA levels under benchmark levels with low effort and providing safe food for consumers.

P2-99 Molecularly Imprinted Solid-Phase Extraction on Microfluidic Chip Coupled with Mass Spectrometry for Rapid Detection of Mycotoxins in Foods

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Introduction: The prevalent occurrence of mycotoxin contamination in various foods and feeds poses health risks to humans and livestock. Current standard analytical methods based on mass spectrometry require sample preparation and clean-up with laborious procedures, relatively high cost and low efficiency.

Purpose: This study aims to develop an automated and low-cost microfluidic chip directly coupled with mass spectrometry to achieve rapid sample clean-up by on-chip molecularly imprinted solid-phase extraction (MISPE) and detection of mycotoxin in foods and feeds.

Methods: The microfluidic chip was fabricated via a two-step polydimethylsiloxane molding followed by micromachinery for installation of the parts. Core-shell molecularly imprinted polymers (MIPs) were prepared using synthesized dummy templates via the sol-gel method. Food samples were homogenized and extracted using 90% acetonitrile with sedimentation for 1 min. Each cycle of on-chip MISPE included 5 steps to achieve repeat use of the microfluidic device: 1) loading MIP, 2) loading sample, 3) washing off interferents, 4) eluting mycotoxin, and 5) unloading MIP and chip cleaning. The eluate was injected into ESI-MS for mycotoxin quantification.

Results: The dummy template, SiO_2 microsphere, and the coated MIPs were successfully synthesized. The static and dynamic adsorption tests confirmed the satisfying performance of the MIP in terms of efficiency (t_{eq} < 5 min) and selectivity (Q_{MIP}/Q_{NIP} > 3). The overall recoveries for different food and feed samples (corn, wheat, oatmeal) at various concentrations (50 ppb - 10 ppm) achieved 72-95% with a limit of quantification < 10 ppb. Each sample took <15 min for the entire test.

Significance: The developed microfluidic chip would significantly reduce the cost and hazardous waste in MS-based mycotoxin detection, benefiting the monitoring of mycotoxin contamination and contributing to food safety.

P2-100 A Bacterial Cellulose Nanocrystal-Based SERS Substrate for Detection of Thiram in Fruit Juices

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Introduction: Thiram as a fungicide has been widely used for controlling fungal diseases in crops, causing the risk to human health due to pesticide residues. Traditional methods for pesticide detection are relatively time consuming, expensive, and require well-trained personal. Thus, developing a simple, rapid, and accurate approach to detect thiram in food matrices is highly demanded.

Purpose: This study aimed to develop a bacterial cellulose nanocrystal (BCNC)-based SERS sensor for detection of thiram in fruit juice.

Methods: The BCNC-based SERS sensor was fabricated by mixing BCNC-SO_3H and AuNPs, followed by dropping and drying on an anodic aluminum oxide membrane. BCNC-SO_3H was used as a stabilizer to support gold nanoparticles (AuNPs) via electrostatic repulsion, fabricating a BCNC-AuNPs SERS substrate with uniformly distributed AuNPs. The prepared SERS substrate was used to detect thiram by a portable Raman spectrometer. The entire test took 12 min including sample preparation and analysis.

Results: This BCNC-AuNPs SERS substrate was utilized to determine thiram residues in peach juice, apple juice, and grape juice, with the limits of detection of 0.036 ppm, 0.044 ppm, and 0.044 ppm, respectively. The detection limits meet the maximum residue levels of thiram in fruit juices required by different countries.

Significance: This BCNC-based substrate is able to serve as a satisfactory SERS sensor for pesticide residue appearing in the food supply chain.

P2-101 Survival, Growth, and Toxin Production of *Bacillus cereus* during Cooking and Storage of Fresh Rice Noodles

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❖ ***Developing Scientist Entrant***

Introduction: Retail stores maintain fresh rice noodles (FRNs) at room temperature because refrigeration negatively impacts FRNs' texture. The room temperature and high aw of FRNs help spore-forming *Bacillus cereus* to grow and produce toxins.

Purpose: This study aimed to investigate the effect of steam cooking on survival and different storage temperatures on the growth and enterotoxins production of *B. cereus* in FRNs.

Methods: The study used three treatments of FRNs: uninoculated, inoculated with 4.0 log CFU/ml, and autoclaved as a negative control, each replicated three times. A slurry of rice flour, cornstarch, and water was steam-cooked for 4 minutes at 90°C, then incubated at 4°C for 168 h and at 22°C and 32°C for 72 h. Incubated FRNs were tested for *B. cereus* growth (plated on MYP agar), pH (AOAC 943.02), and enterotoxin production (BCET-RPLA kit).

Results: Steam cooking reduced the number of *B. cereus* spores by 0.7 log CFU/g. Surviving spores in inoculated and uninoculated FRNs germinated over 72 h of storage. No *B. cereus* was detected in negative controls. Interactions were observed across storage temperatures and time (p<0.05). The *B. cereus* population in uninoculated FRNs increased by over 7.0 log CFU/g at 22°C and 32°C in 72 h. In comparison, inoculated FRNs showed a 5.0 log increase at these storage temperatures due to competitive inhibition from nutrient deficiency at a higher inoculation level. No growth was observed at 4°C in all treatments of FRNs. The pH of inoculated and uninoculated FRNs was reduced from 7.0 to 5.5 at 32°C and 6.3 at 22°C, indicative of FRNS spoilage. *B. cereus* in inoculated FRNs produced enterotoxins after 12 h of storage at 32°C, and over 24 h at 22°C, while no toxin was detected at 4°C.

Significance: FRNs stored at room temperature are only safe to eat within 24 h after preparation; however, further research should investigate the effect of other foodborne pathogens on FRNs.

P2-102 Cytotoxicity Assessment of Psychrotolerant Bacillus cereus Isolates Across Varied Temperatures

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❖ ***Developing Scientist Entrant***

Introduction: The *Bacillus cereus* group, encompassing eight genomospecies such as *B. cereus sensu stricto (s.s.), B. anthracis,* and *B. thuringiensis*, includes members capable of secreting cytotoxic enterotoxins at body temperature (37°C) towards human gut epithelial cells; yet the temperature-dependent growth variations raise uncertainty regarding differential toxin expression despite the presence of virulence genes.

Purpose*:* The objective of this study was to assess differences in cytotoxicity among psychrotolerant *B. cereus* isolates belonging to clades II and VI when grown at 32°C and 37°C through *in vitro* cytotoxicity experiments.

***Methods**:* 70 isolates belonging to two phylogenetic groups (group II and VI) were grown in brain heart infusion (BHI) broth at 32°C and 37°C to collect cell-free supernatants. Cytotoxicity was assessed using the WST-1 assay and human colorectal carcinoma (CaCo-2) cells. CaCo-2 were exposed to 15% v/v of cell-free bacterial supernatants for 15 minutes before adding the WST-1 dye and allowing for dye conversion, which indicates cellular metabolic activity. The absorbance data was min-max normalized to BHI and *B. cereus* ATCC 14579 to determine cytotoxicity. The difference between cytotoxicity values at 32°C and 37°C was compared within clades using a one-way ANOVA with a Tukey post-hoc test.

***Results**:* Isolates from group II (n=33) had an average cytotoxicity of 0.3758 ± 0.0873 when grown at 32°C and 0.3448 ± 0.0316 at 37°C (p=0.6078). Isolates from group VI (n=37) had an average cytotoxicity of 0.2870 ± 0.1157 when grown at 32°C and 0.2268 ± 0.0351 at 37°C (p=0.3490). Notably, 10 isolates grown at 32°C from group II (n=4) and group VI (n=6) had significantly higher cytotoxicity values (p=0.0060 and $2.5306*10^{-5}$) but were not found to be cytotoxic when evaluated at 37°C.

Significance: While some isolates differed in cytotoxicity when grown at 32°C compared to 37°C, differences were not significant when comparing all isolates within group II and VI.

P2-103 L-Alanine and Phenylalanine Act Synergistically to Induce Germination in *B. cereus* Biovar Thuringiensis and Non-Thuringiensis

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Introduction: *Bacillus cereus sensu stricto* (*s.s.*) is one of the eight genomospecies within the *Bacillus cereus* group, which contains strains capable of causing foodborne illness, as well as strains that produce insecticidal crystal proteins (biovar Thuringiensis). While *B. cereus s.s.* biovar Thuringiensis (Bt) has traditionally been utilized as a non-toxic biocontrol agent, safety concerns have been raised regarding Bt strains that express enterotoxins. However, it is unclear whether Bc and Bt strains have similar ability to survive gastrointestinal (GI) tract stresses and germinate, which is a prerequisite for human toxicoinfection.

Purpose: This study aimed to evaluate the impact of human GI tract-related stresses and nutrients on the germination of *B. cereus s.s.* biovar Thuringiensis and non-Thuringiensis strains.

Methods: Sixteen isolates (8 biovar Thuringiensis, 8 non-Thuringiensis) from diverse sources were subjected to simulated oral, gastric, and duodenal conditions. Isolates grown on T3 agar for 4 days were used to prepare spore suspensions. After simulation, surviving *B. cereus s.s.* cells were enumerated to identify the amount of germination. Additionally, various potential germination inducers and amino acids were tested, and optical density (OD_{600}) changes were monitored over time to estimate germination levels. The significance of differences in the effects of different germination inducers were assessed using Kruskal-Wallis test.

Results: Exposure to GI tract-related stresses resulted in insignificant spore germination ($p = 0.66$). In contrast, specific nutrients, such as inosine (p = 4.0-12), and L-alanine combined with L-phenylalanine ($p = 0.03$), significantly induced germination. Notably, there were no discernible differences in spore germination between *B. cereus s.s.* biovar Thuringiensis and non-Thuringiensis strains across all treatments ($p > 0.05$).

Significance: The findings emphasize the importance of considering specific nutrient conditions, such as inosine and L-alanine + L-phenylalanine, when assessing the potential germination of *B. cereus s.s.* spores in the human GI tract.

P2-104 Isolation and Characterization of *Bacillus cereus* Strains Isolated from Various Food Products in Mali

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Introduction: *Bacillus cereus sensu lato* or *B. cereus* group is composed of ubiquitous and endospores forming bacteria. The ingestion of contaminated and *unrefrigerated* food containing *B. cereus* strains can cause foodborne illnesses. Thus, *B. cereus* can cause two types of food-borne diseases: The diarrheal or the emetic syndromes. Fatal episodes of *B. cereus* foodborne illness have been recorded around the world. In Mali, there is a scarcity of data on the detection of *B. cereus* in food products.

***Purpose**:* The objective of this study was to isolate and characterize *B. cereus* strains from various food products in Mali.

***Methods**:* Sampling of food products was implemented in Malian stores and open markets. The ISO 7932:2004 norm have been adapted to detect and isolate *B. cereus* strains from food samples. Each sample was inoculated on MYP agar and hemolysis tests were performed on 5% of sheep fresh blood agar. All *B. cereus* strains were screened for toxin genes by PCR.

Results: A total of 30 food products, including raw rice and spices, were investigated. Following an enrichment step, *B. cereus* was detected in all tested samples. Indeed, selecting two to five characteristics colonies per positive sample, a total of 132 *B. cereus* strains were isolated. Unsurprisingly, they were all beta-hemolytic on blood agar. The *cytK-2*, *nheD* and *hblA* genes were respectively present in 79.55%, 43.94% and 5.30% of the tested strains. The *EM* gene which codes for emetic toxin was only detected in three strains originating from a raw rice sample. As for *cytK-1*, it was not detected in any of the strains.

Significance: In Mali, a developing country with potentially failing food hygiene where rice is used as main menu, *B. cereus* could be a threat to malian health. These results give a first insight on this pathogen and its toxin gene distribution in some strains isolated from local food products.

P2-105 Inactivation of *Clostridium botulinum* Spores in Commercial Cold Brew Coffee Stored Under Aerobic Conditions

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Introduction: Unlike traditional hot brew coffee, cold brew coffee is prepared by brewing the coffee grounds at ≤25°C for approximately 8 to 36 hours. Since brewing temperature greatly affects the aqueous solubility of compounds and the chemical composition, the antimicrobial activity of cold brew coffee extracts likely differs from that of traditional hot brew coffee. In the absence of heating, cold brewed coffee may have a greater microbial safety risk. In previous studies focused on botulinum toxin formation, we found cold brew coffee stored under aerobic conditions may inactivate *C. botulinum* spores.

Purpose: The purpose of this study is to determine the effects of cold brew coffee storage conditions on *C. botulinum* spore populations over time.

***Methods*:** A 10-strain cocktail of proteolytic *C. botulinum spores (*5 Type A and 5 Type B strains) was heat-shocked (80°C for 10 minutes) and inoculated into 10 mL of cold brew coffee at a concentration of 10^3 spores/ml (in triplicate). Inoculated samples were stored long-term (up to 3 months) at 27°C under anaerobic and aerobic conditions. The aerobic and anaerobic coffee samples were enumerated every 7 days for 28 days using the 5-tube MPN method with Trypticase-Peptone-Glucose-Yeast (TPGY).

***Results*:** The *C. botulinum* spore concentration of the cold brew coffee stored under anaerobic conditions remained at 10^3 spores/ml during the entirety of a 28-day storage. In contrast, the spore concentration of the aerobically stored cold brew coffee gradually decreased from 10^3 spores/ml at day 0 to <1.8 x 10^0 spores/ml after 28 days.

Significance: This study will determine whether cold brew coffee stored under aerobic conditions inactivates *C. botulinum* spores and will provide the foundational data for future work to identify antimicrobial chemical properties in cold brew coffee using an untargeted HPLC-Q-TOF-MS approach.

Poster

P2-106 Assessment of the Applicability of *Bacteroidales* Molecular Markers Described in the Scientific Literature for Microbial Source Tracking (MST) in Chilean Fecal Samples

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Undergraduate Student Award Entrant

Introduction: *Bacteroidales* molecular markers are used for microbial source tracking (MST) to identify and quantify host-specific contamination between different species, employing qPCR (quantitative polymerase chain reaction) in surface waters. However, the challenge lies in the genetic variability of these bacteria, which depends on the region. It is important to evaluate and optimize a protocol in Chile, as they are not described.

Purpose: This study focused on evaluating the specificity of molecular markers of *Bacteroidales* in Chilean fecal samples, including bovine, canine and human samples, using qPCR.

Methods: A review of 100 scientific articles was conducted, where molecular markers of Bacteroidales (primers and probes) for the target species were selected based on pre-defined inclusion and exclusion criteria. Total DNA extraction was performed from fecal samples of the target species using the E.Z.N.A Stool DNA kit (Omega). Molecular markers for each species were identified through endpoint PCR using the extracted DNA from the sample of the respective species as the target. Subsequently, the specificity of the selected probes was determined using qPCR, with the target being the DNA extracted from all the species of interest.

Results: All selected molecular markers (bovine, canine, human, and universal) from the literature were detected through endpoint PCR in their respective DNA samples. The identification of *Bacteroidales* through probes in qPCR showed a sensitivity of 83.3% (5/6) in bovine and 66.7% (4/6) in canine samples; No cross-amplification was observed with the other species; therefore, both probes exhibited 100% specificity (12/12). On the other hand, the probes for humans and universals did not generate amplification in qPCR.

Significance: This study emphasizes the importance of carefully selecting molecular markers for the identification of fecal contamination sources by qPCR. This allows us to subsequently design mitigation measures to reduce fecal contamination in water used for agricultural purposes.

P2-107 Characterization of Antibiotic-Resistant Lactic Acid Bacterial and Pathogenic Isolates from Retail Kimchi

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Developing Scientist Entrant

Introduction: Consumption of fermented foods has been encouraged recent years by the mainstream media to improve or replenish gut microbiota. However, our previous study found that dietary intervention by fermented foods resulted in a significant surge of the antibiotic resistome in human gut microbiota, and antibiotic resistant bacteria isolates were highly prevalent in most retail kimchi products.

Purpose: This study assessed representative antibiotic resistant bacterial isolates from kimchi, examining potential molecular mechanism(s) that align with their resistance profiles.

Methods: Nineteen pre-screened suspicious kimchi isolates were challenged using microdilution method and commercial antimicrobial susceptibility Sensititre® kits for the spectrum of resistance against clinically relevant antibiotics. Multidrug resistant isolates with specific features of interest underwent whole-genome shotgun sequencing assessment. The sequencing data were cleaned with fastp tool, assembled with MEGAHIT and analyzed with CARD RGI online platform.

Results: Multidrug resistant *Klebsiella pneumoniae* is problematic due to the involvement in nosocomial infections. Multiple antibiotic resistance genes, encoding various resistance mechanisms, i.e., efflux pumps, porin protein reducing permeability of antibiotics, SHV β-lactamase for extended-spectrum of β-lactam antibiotics, were found co-existing in *K. pneumoniae* K7-6, rendering resistance to antibiotics ranging from macrolides to β-lactams. *Weissella sp.* K5-3, a lactic acid bacterium able to drive kimchi fermentation and survive the low-pH in fermented food matrices, was also worth-noting for its high resistance to the whole Sensititre® panel.

Significance: Besides the potential dissemination of antibiotic resistance genes, foodborne multidrug resistant pathogenic and lactic acid bacterial isolates also represent a significant public health risk for infections such as bacteremia and sepsis, resistant to antibiotic treatment, particularly in susceptible patients with damaged gastrointestinal tracts and compromised immune functions who may seek gut microbiome rescue. This study calls for more comprehensive investigations into the health impact of traditionally fermented foods and targeted mitigation of foodborne antibiotic resistance.

P2-108 Effect of Application of Smoke System on Pre- and Post-Grinding Stage and Impact on Shelf Life of Fresh Ground Poultry

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Introduction: Smoke system technology offers clean label solutions for meat shelf-life and food safety extension and imparts positive product characteristics such as flavor, color and preservation impact.

Purpose: To evaluate the stage of the application of smoke technology in the process as means to maximize shelf-life in ground poultry.

Methods: The smoke system Cloud S-6 at 0.5% and 1.5%, and Cloud S-9 at 0.5% and 1.0%, were used to treat fresh boneless chicken thighs. Two application methods were tested. First, smoke system was sprayed on the chicken thighs followed by grinding. Second, smoke systems were directly added to the ground poultry. Treated samples were packed under aerobic atmosphere and stored at 4 °C for up to 25 days. At each sampling, duplicate samples were processed and plated . Differences among the treatments were determined using one-way ANOVA using JMP Pro version 16.1.0 (SAS Institute Inc., NC, US), with significance set at $P<0.05$.

Results: Cloud S-9 was more effective in controlling the LAB and APC in fresh poultry as compared to Cloud S-6. As expected, higher concentrations of Cloud S-6 (1.5%) & Cloud S-9 (1.0%) showed lower microbial populations irrespective of the application method. For APC, Cloud S-9 improved the shelf-life of ground poultry by 25% to 8 days as compared to the control (no preservative) which reached spoilage threshold (6 log CFU/g) by 6-days. Similarly, all smoke treated samples had significantly ($p< 0.05$) lower LAB population (4.6–5.4 log CFU/g) than control which reached spoilage limit on day 6 of storage. Interestingly, no significant differences ($p>0.05$) were observed between bacterial population among the two smoke application methods.

Significance: Pre-or post-grinding stage application of smoke system exhibits similar efficacy for shelf-life of fresh poultry.

Poster

P2-109 Enhancing Fresh Poultry Shelf Life: Evaluating the Role of Natural Flavor in Spoilage Control and Oxidation Delay

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Introduction: Addition of clean label natural flavor system to traditional vinegar could extend the shelf life of fresh poultry while delaying the onset of oxidation.
Purpose: To assess the antimicrobial and antioxidant efficacy of natural flavor fractions.
Methods: Ground chicken was treated with 1. 1.0% Natural flavor (Innovation component 1; IC1); 2. 0.75% vinegar (Innovation component 2, IC2); 3. Combination of IC1 (1.0%) + IC2 (0.75%). Treated samples were stored under aerobic atmosphere at 4°C for 30 days. On each sampling day, duplicate samples were enumerated for lactic acid bacteria (LAB) and aerobic plate count (APC). Spoilage threshold was considered as 6 log CFU/g. To assess the impact of IC1 in delaying oxidation, samples were analyzed for the concentration of malondialdehyde (MDA) via TBARS assay at days 0, 12, and 25. Differences among the treatments were determined using one-way ANOVA at $P< 0.05$.
Results: The vinegar (IC2) at 0.75% effectively controlled growth with APC and LAB counts below 6 log CFU/g until day 14, significantly ($p<0.05$) surpassing control (no preservative), which spoiled by day 7. For APC, natural flavor (IC1) was effective in extending the poultry shelf-life to 9 days. The combination of IC1 (1.0%) and IC2 (0.75%) further extended the number of days to reach spoilage limit to 18 days. Based on TBARS assay, MDA concentration rose for all treatments from day 0 to 25, except for IC1 which showed lowest increase (0.85 µg/g) compared to control (1.6 µg/g) by the end of the shelf-life.
Significance: Natural flavor and vinegar systems substantially extend the shelf-life and quality of fresh poultry while mitigating oxidation stress.

P2-110 Synergistic Effect of Natural Flavor and Vinegar for Shelf-Life Extension of Fresh Poultry

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Introduction: Natural flavor and weak organic acids-based preservation system provide clean label solutions for meat shelf-life and food safety extension.
Purpose: To evaluate the efficacy of natural flavor (Innovation concept 1; IC1) individually and in combination with vinegar (Innovation concept 2; IC2) for control of spoilage microorganisms in ground chicken.
Methods: Ground chicken was treated with 1.5% IC1 alone and in combination with 0.75% IC2. Treated samples were packed under aerobic atmosphere and stored at 4 °C for up to 30 days. On each sampling day, duplicate samples were processed to enumerate for lactic acid bacteria (LAB), aerobic plate count (APC), Enterobacteriaceae (EB) and *Pseudomonas*. Spoilage threshold was considered as 6 log CFU/g. Differences among the treatments were determined using one-way ANOVA using JMP Pro version 16.1.0 (SAS Institute Inc., NC, US), with significance set at $p \leq 0.05$.
Results: IC1 in combination with IC2 was effective in controlling LAB, APC, EB, and *Pseudomonas* in ground chicken at 4°C. IC 1 alone provided control of APC, EB, and LAB until days 8, 9, and 10 while untreated control reached 6 log CFU/g by day 7. Treatment of IC1 (1.5%) combined with IC2 (0.75%) exhibited synergistic effect on ground chicken, significantly enhancing the shelf life to 20 days (p <0.05) while IC2 alone provided 14 days of shelf life. All the combination treatments controlled the EB and *Pseudomonas* population for more than 20 days.
Significance: Combination of Kerry's natural flavor and vinegar-based preservation systems offers a promising clean label solution for shelf-life extension of ground poultry while meeting consumer expectations for safer food products.

P2-111 Impact of Alginate on Probiotic Growth and Butyric Acid Production

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❖ ***Developing Scientist Entrant***

Introduction: Butyric acid, produced by beneficial gut bacteria, offers numerous health benefits, including improved digestion, enhanced nutrient absorption, immune system regulation, inflammation reduction, harmful bacteria inhibition, and metabolic health promotion.
Purpose: This study aimed to analyze the effects of alginate as a prebiotic on the growth rates and butyric acid production of four probiotic microorganisms within the gut.
Methods: We incubated the four probiotic microorganisms (*L. acidophilus, L. gasseri, L. fermentum, L. bulgaricus*) at 37℃ for 24 hours in MRS (de Man, Rogosa, and Sharpe) broth, with or without the presence of 25mg/ml of alginate. Growth curves were generated by monitoring optical density at 600nm, and butyric acid production was quantified using a General Butyric Acid (BA) ELISA Kit.
Results: After 24 hours of culturing, the addition of alginate resulted in a significant increase in the proliferation of *L. acidophilus, L. gasseri, L. fermentum, and L. bulgaricus*, showing a respective 0.6, 2.3, 2.17, and 2.33-fold growth compared to the control group. Furthermore, when the supernatant from the alginate-treated cultures of *L. acidophilus*, *L. gasseri*, *L. fermentum*, and *L. bulgaricus* was collected and compared to the control group after 24 hours of incubation, it revealed a respective 0.7, 1.28, 2.54, and 5.9-fold increase in butyric acid levels.
Significance: This knowledge can be applied to promote gut health, leading to improved digestion, nutrient absorption, and immune regulation. It has practical applications in dietary interventions and offers opportunities for the healthcare and nutraceutical industries to develop products aimed at enhancing gut health and overall well-being.

P2-112 Analysis of Spoilage Microorganisms from Food and Environment Samples by IR Biotyper®

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Introduction: Hygiene control and source tracking throughout food and beverage production lines requires several steps, from surface sampling to molecular analyses. Microbial strain typing for source tracking and quality control is an essential step in maintaining food product safety. Fourier Transform - Infra-Red (FT-IR) spectroscopy system, the IR Biotyper®, offers an easy-to-use method for fast and effective microorganism strain typing.
Purpose: This study aimed to explore the IR Biotyper® for strain typing and analysis of the spoilage microorganisms isolated from food and environment samples in a plant.
Methods: A number of 65 isolates including lactic acid bacteria (LAB) and yeasts isolated from finished product samples, environmental swabs and spoilage microorganisms were analyzed by the IR Biotyper®. The identification of microorganisms was obtained by analysis via MALDI Biotyper sirnus one system before running on IR Biotyper®. The dendrogram and 2D/3D scatter plot were displayed. Different dimension reduction parameters were used for analysis.
Results: More than 900 spectra were generated from runs on IR Biotyper® with 649 spectra from yeasts and 269 from LAB. The software analyzed IR spectra in the wavenumber range for polysaccharides, as well as other regions such as those indicating fatty acids and proteins. Dendrogram using Euclidian distance and average linkage clustering method were generated to show the spectral relations. For example, Cut-Off Value (COV) was optimized at 0.511 to allow discrimination on all *Lactobacillus plantarum* isolates while maintain coherence (correlation coefficient 0.986) within the cluster at wavelength 1300-800/cm. The 3D scatter plot (using 9 PC representing 95.5% variance), isolates coming from different sources or locations were well separated

from each other.

Significance: With a discriminatory power comparable to routine molecular genetic methods, the IR Biotyper® meets the needs for fast screening, allowing for real-time quality control and source-tracking. It could be a potential tool for spoilage risk assessment and enhance spoilage investigations.

P2-113 Comparison of Different Production Processes for Buffered Peptone Water for the Microbiological Examination of Food Regarding Time, Energy and Water Requirements

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Introduction: Quantifying resources needed per food analysis and an independent comparison of the manufacturing processes is helpful for the QC companies to holistically assess the efficiency of the various media preparation methods.

Purpose: Comparison of different production processes for buffered peptone water for microbiological examination of food in terms of time, energy, and water requirements, performed by an independent food QC laboratory.

Methods: Five different media manufacturing methods were compared, and process throughput times plus hands-on times of the employees were determined (ReadyStream® system classic and in combination with Dilutor, classic media production in the laboratory, use of a media preparator and ready-to-use media bags). Steps of media production, preparation of first dilution of the samples, and preparation and follow-up steps (e.g. cleaning of the instruments) were considered. Reference value was 100 L of buffered peptone water and activity duration of 20 samples 25g each and 10 sample weights of 375g were documented.

Results: Process time is highest for 25g and 375g samples with classic media production (25g: 145.1 sec/375g: 1875.4 sec) and lowest for 25g sample with media bags (60.5/506.0) directly followed by media preparation system without (66.8/273.2) and with dilutor (65.7/620.4). media preparation system with dilutor gave the shortest hands-on time (25g: 56.5 sec) and classic for 375g samples media preparation system (233.8). Looking at the energy consumption and water consumption per sample, the mediabags showed the lowest values (25g: 0.0007 kWH/0.0L H2O) and media preparation system takes 2nd place (e.g. 0.005 kWH and 0.23 L for 25 g probe). Manuel production and media preparator gave the highest values in all comparisons.

Significance: Comparison of manufacturing processes gives laboratories the opportunity to calculate their efficiency and reduce laboratory costs without increasing the analytical costs for food producers. This preserves the number of food inspections and thus contributes to food safety.

P2-114 Reducing the Risk of Temperature out of Specification of Using Pre-warmed Enrichment Broth for Enrichment Delivery and Incubation

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Introduction: Enrichment Broth is prewarmed and held at room temperature prior to sample addition introducing potential risk that the broth may be below temperature specification at the time of delivery into the sample.

Purpose: This study demonstrates and compares the characterization of prewarmed broth temperature from initial warming through use in sample preparation using traditional media preparation and delivery verses the ReadyStream® system.

Methods: Enrichment broth temperature was monitored and collected every 15 minutes. Room temperature broth was prewarmed in a 37°C incubator or prepared using the media preparation system. Prewarmed broth was removed and monitored over 3 hours to characterize temperature loss for prewarmed broth held at room temperature. Once the holding time was up, broth was immediately added to pre-weighed samples, 80% ground beef, and bags homogenized for 2 minutes. Prewarmed broth from the media preparation system was combined with ground beef samples and homogenized and the temperature monitored.

Results: Smaller volumes and longer holding times at room temperature led to lower starting temperatures. 225ml at 3 hr. with starting temperature of 21°C compared to 3375 ml media prep system with starting temperature of 34°C. The average temperature over the incubation period is lower for samples where the broth was left out longer (at 19.5 hr. 31°C vs. 35°C). Over an 8–10 hour incubation period, samples that sat out for long periods at room temperature had average incubation temperatures below the 37°C set temperature of the incubator ranging 28 – 33°C compared to 33 – 36°C with media preparation system.

Significance: Keeping enrichment broth at controlled temperature specifications is critical to reduce risk of achieving optimal results, especially for shorter incubation times. Many procedures call for use of prewarmed media do not allow for the cooling factor after the sample has been added; therefore, creating a potential for false negatives since target pathogens may be incubated under optimal conditions, thus introducing safety risks.

P2-115 Antibiotic Resistance in Gram-Positive and Gram-Negative Bacteria Isolated from Street-Vended Foods and Fresh Vegetables in Sylhet City

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illness is caused by a foodborne pathogen that comes from street vended foods and fresh vegetables, and it poses a great threat to human health because it is identified as a potential vector for the spread of disease.

***Purpose*:** To evaluate the antibiotic resistance patterns of bacterial isolates collected from street-vended foods (phuchka, chatpati) and salads in Sylhet city, Bangladesh.

Methods: Biochemical and culture identification of the collected isolates (n=15) was done by colony characteristics and the API (Analytical Profile Index) 20E test. Then their susceptibility to 11 different antibiotics was investigated by the Kirby-Bauer disk diffusion method.

***Results*:** We found a high prevalence of bacteria isolates in street-vended foods and fresh vegetables (53.33%) exhibiting multidrug resistance. The multiple antibiotic resistance (MAR) index showed that 8 isolates had MAR above 0.25. All isolates were sensitive to ceftriaxone, a third-generation cephalosporin. About 100% of *E. coli*, *Klebsiella*, and *Staphylococcus aureus* strains were resistant to oxacillin, and one strain of *E. coli* was extensively drug-resistant (XDR).

Significance: The study raises concern about the safety of street-vended foods and fresh vegetables and warns about the lesser-known transmission of resistant bacteria to humans.

P2-116 Risk Associated with Preservation Methods on Toxigenic Fungi and Their Mycotoxins in Dried Fish Sold in Markets in Gauteng, South Africa

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Introduction: Dried fish play a significant role as dietary protein and a source of income in South Africa. The presence of mycotoxins, produced by pathogenic fungi during fish processing, poses a considerable risk to consumer health.

Purpose: This study evaluated potential health risks associated with toxigenic fungi and their mycotoxins production in imported dried fish, sold in both formal and informal markets in Gauteng, South Africa.

***Methods*:** A total of 115 dried fish samples were randomly selected, including sundried (48), salted (20), and smoked (47) variants, stored either frozen

or non-frozen. Using potato dextrose agar (PDA) plate purification, and internal transcribed spacer (ITS) sequence analysis. Aflatoxins content in fish has been determined using High-performance liquid chromatography (HPLC).

Results: It was observed that smoked dried fish exhibited a higher overall fungal contamination rate (63.8%; n=30; p<0.001) compared to salted (35%; n=7) and sundried (29.2%; n=14) samples. The study isolated and identified 31 fungal strains from dried fish with *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. being the three predominant genera. HPLC analyses detected mycotoxin residues in 25 out of the 115 (21.7%) dried fish samples. Aflatoxin B_1 was the most frequently detected and the concentration ranged from 0.02 to 3.27 µg/kg with highest level observed in smoked dried fish samples. Aflatoxin B_2, AFG_1, and AFG_2 concentrations ranged from 0.11 to 1.43, 0.03–3.20 and 0.61 µg/kg respectively.

Significance: The substantial occurrence of fungal populations and mycotoxins in dried fish within the Gauteng market raises concerns about potential threats to consumer health. It is recommended that future processing methods incorporate advanced techniques and controlled storage conditions to minimize, and ideally eliminate, fungal contamination during dried fish processing.

P2-117 Exploring the Fundamental Research Path of *Aeromonas hydrophila* Contamination in Food and Charting Future Direction

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Introduction: *Aeromonas hydrophila*, a prevalent Gram-negative bacterium, poses a substantial threat to food safety due to its inherent capability to form resilient biofilms within the food industry. This study underscores the challenges inherent in *A. hydrophila* contamination in food and emphasizes the imperative for comprehensive research to address this looming concern.

Purpose: The purpose of the study is to evaluate the efficacy of probiotics and chemical agents and conduct a bibliometric analysis to access the prevalence, distribution, and inhibitory strategies related to *A. hydrophila* contamination in food.

***Methods*:** Conducting a thorough bibliographic study utilizing the Vosviewer software, we explore the prevalence of *A. hydrophila* in the food matrix. Recent methodologies, incorporating advanced microscopy, elucidate the impact of probiotics and chemicals in mitigating *A. hydrophila* growth. Furthermore, fundamental characteristics, such as time-dependent growth, pH variations, and biofilm-forming ability, have been assessed.

Results: A bibliometric analysis of *A. hydrophila* biofilm research reveals significant knowledge gaps. The average biofilm formation for ATCC 15467 across various materials is highest on fish fillet (6.02 Cfu/cm²) and lowest on stainless steel (5.00 Cfu/cm²), while for KCTC 2358, it is highest on fish fillet (6.28 Cfu/cm²) and lowest on stainless steel (5.33 Cfu/cm²). Additionally, fusel oil demonstrates a minimal inhibitory concentration (MIC) of 0.08%, underscoring its efficacy in inhibiting biofilm formation which was visualized by confocal images. Furthermore, the supernatants from both probiotic bacteria exhibit superior capabilities in inhibiting *Aeromonas* biofilm formation. Specifically, the supernatant from D7 reduces biofilm formation by 31.2%, while the supernatant from B67 achieves a reduction of 39.4%.

Significance: This research, employing bibliometric analysis, crucially identifies knowledge gaps in *A. hydrophila* biofilm studies. Emphasizing the imperative for targeted interventions, it reveals strain-specific biofilm-forming capabilities and highlights the inhibitory potential of Fusel oil and probiotic supernatants, contributing to enhanced food sector safety.

P2-118 Efficacy of Chitosan-Based Coating on Survival of *Salmonella* spp. on Reusable Plastic Containers and Mold Inhibition on Blueberries

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❖ ***Developing Scientist Entrant***

Introduction: Blueberries *(Vaccinium corymbosum)* are the second-most produced and economically important soft fruit in the United States. They are very perishable, consumed raw and serve as potential reservoirs for pathogens such as *Salmonella spp*.

Purpose: To evaluate the effectiveness of chitosan-based coatings on *Salmonella spp.* on reusable plastic containers (RPC) comparable to the harvest containers used during harvesting and post-harvesting processes. Furthermore, to assess the efficacy of the chitosan-based coatings was evaluated during a short shelf-life observation of blueberries by potential reducing yeast and mold growth.

Methods: Four chitosan-based coatings were formulated to coat the RPC and blueberries for both objectives. Samples were randomized in triplicate to maintain consistency when analyzing *Salmonella spp.,* moisture loss, yeast, and mold, and scanning electron microscopy.

Results: The results of the treatments (no coating, chitosan only, carvacrol only, and chitosan + carvacrol) and sampling time (0, 4, 8, 12, and 24 h) were affected by the interaction ($p < 0.05$) between treatment and time. After 12 h, the treated samples (carvacrol, chitosan, and chitosan + carvacrol) completely inhibited *Salmonella spp.* on the RPC. Initial counts were below the limit of detection, therefore, an enrichment with Rappaport Vassiliadis broth (R.V.) was performed and confirmed the absence of *Salmonella spp.* The chitosan-only treatment was effective in suppressing the growth of yeast and mold to 1.8 log CFU/g and 2.8 log CFU/g respectively.

Significance: This research provides a practical method in the application of chitosan coatings to inhibit *Salmonella spp.* and suppress the growth of mold and yeast.

P2-119 Characterizing Effects of Short Chain Fatty Acids on *Campylobacter jejuni* Virulence Factors

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Introduction: *Campylobacter jejuni* is a leading cause of bacterial gastroenteritis worldwide, clinically manifesting with either bloody/inflammatory or watery diarrhea. In this study, we aimed to investigate the effects of short chain fatty acids (SCFAs) on the virulence and physiology of *C. jejuni* strains associated with different clinical diarrheal manifestations.

Purpose*:* SCFAs are a critical component of the intestinal tract, therefore understanding the impact these factors have on *C. jejuni* pathogenicity is essential for developing therapeutic and preventative measures.

Methods*:* Ten *C. jejuni* strains (five associated with watery diarrhea and five with bloody/inflammatory diarrhea) were used for different assays. Growth curves and attachment assays with Caco-2 intestinal epithelial cells were conducted with each of the ten strains with either no SCFAs present, sodium butyrate, or sodium acetate to determine the impact on growth and intestinal cell attachment.

Results*:* Our results indicate butyrate has a statistically significant impact on the attachment of bloody/inflammatory strains ($p = 0.0434$), but not on those strains associated with watery diarrhea manifestations as a group ($p = 0.359$). However, individually the watery associated strain RM1221 was highly statistically altered for attachment in the presence of butyrate ($p < 0.001$), while the other four were not significantly impacted. Although the presence of butyrate did not significantly alter the growth rate of *C. jejuni* strains associated with either diarrheal manifestation. However, sodium acetate did result in a significant change in the growth rate of strains associated with bloody/inflammatory diarrhea manifestations ($p = 0.0199$) but not watery diarrhea manifestations ($p = 0.746$).

Significance: Therefore, our data indicates that butyrate and acetate have more of an impact on strains associated with bloody/inflammatory diarrhea manifestations than those strains associated with watery diarrhea manifestations. Understanding the effects of SCFAs on *C. jejuni* virulence, particularly those associated with more severe disease that could result in the need of treatments will assist in developing new therapeutic strategies.

Poster

P2-120 Exploring the *Campylobacter* Dormancy States Triggered by Stressors in Agri-food Systems and the Dynamic Shift from Light to Deep Dormancy

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Introduction: *Campylobacter jejuni* is a foodborne pathogen and one of the most common bacterial agents of human diarrheal diseases. Despite its sensitivity to environmental stresses, this microbe is ubiquitously distributed throughout the food production chain. Dormancy plays a vital role in the survival of *C. jejuni*, particularly in adverse environmental conditions. *C. jejuni* has been shown to exhibit two stress-tolerant dormancy states: antibiotic persistence and viable but nonculturable (VBNC).
Purpose: Considering the challenges in controlling *C. jejuni* in the agri-food system, this research project focused on investigating the role of food-related environment in inducing dormant states of *C. jejuni* and how this dormancy contributes to the survival of *C. jejuni* during food processing and storage.
Methods*:* Different *C. jejuni* strains were incubated under starvation and high salt conditions. Persister cells were quantified by subjecting a bacterial population to a lethal concentration of antibiotics, which eliminates most actively growing cells. VBNC *C. jejuni* cells were quantified by propidium monoazide (PMA)-quantitative polymerase chain reaction (qPCR) coupled with the plating assay. The morphology changes of *C. jejuni* cells under different stressors were observed by transmission electron microscopy (SEM). All experiments were conducted in at least triplicate.
Results: The normal and persistent subpopulations under the antibiotic treatment evolve over time according to two exponential patterns. The persister fraction in the initial population of *C. jejuni* was estimated to be ~0.29%-0.65%. Around 3% of persister cells entered the VBNC state after incubation in 7% NaCl for 24 hours. The VBNC *C. jejuni* also showed significant morphological changes compared to actively growing cells, specifically the stressed cells became coccoid rather than spiral-shaped.
Significance: Understanding the dormancy continuum of *C. jejuni* can lead to novel approaches to reduce its prevalence and persistent contamination, not only the agri-food system but also in clinical practices and other One Health-related fields.

P2-121 Methylsulfonylmethane Inhibits *Salmonella enterica T*yphimurium, Enteritidis and Infantis in a Bacteriostatic Manner

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Introduction: Methylsulfonylmethane (MSM) has been shown to inhibit growth of *Escherichia coli* and *Salmonella* Kinshasa. The Food and Drug Administration (FDA) has classified MSM as Generally Regarded as Safe (GRAS). The Food Safety and Inspection Service (FSIS) has identified three *Salmonella* serotypes as key performance indicators for evaluating success in reducing *Salmonella* contamination: Infantis, Enteritidis, and Typhimurium.
Purpose*:* This study evaluates antibacterial properties of MSM against *Salmonella enterica* serotypes Infantis, Enteritidis, and Typhimurium.
Methods*:* Bacterial proliferation analysis was measured spectrophotometrically during log phase growth with 0, 5, 10 and 16% MSM. To assess the mechanism of inhibition, cultures were grown overnight with 0, 5, 10 and 16% MSM and enumerated on unmedicated Brain Heart Infusion Agar (BHIA) or BHIA with 0, 5, 10 and 16 % MSM. The long–term viability studies were done to evaluate the impact of 10% MSM.
Results*:* Absorbance data indicated a dose–dependent inhibition from 0–16% MSM. There was no growth of Infantis, Enteritidis or Typhimurium BHIA in 10 or 16% MSM. Both strains enumerated on unmedicated BHIA from overnight cultures with 10 or 16% MSM were able to partially recover. Recovery after MSM removal may be indicative of a bacteriostatic mechanism of inhibition. The long–term viability studies illustrated that none of the *S. enterica* serovars could be rescued from 10% MSM after 5 days, respectively.
Significance: MSMs antibacterial activity may prove useful during pre– or post–harvest food safety as a disinfectant, the primary benefit being its clinical safety to humans. Comparisons to other disinfectants would also need to be made to determine if MSM was superior to those already on the market and would be cost effective.

P2-122 Positively Charged Micro-Nanoplastics Induce ARG Expression in *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: The coexistence of antibiotic resistance genes (ARGs) and micro-nanoplastics (MNPs) is a significant public health concern. MNPs help form bacterial communities, absorb pollutants, and potentially act as vehicles for ARGs. While limited information exists on MNP-dependent ARG expression, our study investigated the effects of differentially charged polystyrene (PS)-MNPs on *Salmonella enterica* by assessing growth, cell density, and ARG expression.
Purpose: This study aimed to investigate the impact of varying concentrations of oppositely charged MNPs on the growth and antimicrobial resistance (AMR) profile of *Salmonella enterica*.
Methods: A clinical isolate of *Salmonella enterica* was cultured in LB broth for 24 hours, exposed to +ve/-ve surface-charged PS-MNPs at 25 and 100 ppm concentrations, and treated with antibiotics (penicillin, chloramphenicol, sulfamethoxazole, azithromycin, kanamycin) at concentrations from 2 to 512 µg/ml using the broth dilution method for 72 hours. OD at 600 nm was recorded, and real-time PCR assessed the gene expression of 8 ARGs (*blaTEM*, *cm1A*, *sull*, *ermB*, *kn*, *acrA*, *marA*, *sdiA*) relative to the housekeeping gene *rpoD*.
Results: In the broth dilution assay, exposure to positively charged PS-MNPs and antibiotics resulted in a higher OD at 600 nm, indicating increased cell density. Positively charged PS-MNPs induced significant upregulation (6.07 log-fold change) of *blaTEM* at 100 ppm, while negatively charged PS-MNPs showed a minimal change (0.16 log-fold). Additionally, 100 ppm of positively charged PS-MNPs led to substantial upregulation (6.01, 5.53, 5.42 log-fold changes) in *acrA*, *marA*, and *sdiA*, while negatively charged PS-MNPs caused downregulation (0.91, 0.69, 0.25, 0.29 log-fold changes) in *cm1A*, *sull*, *kn*, and *ermB*.
Significance: This study on charged MNPs and drug resistance in *Salmonella enterica* provides critical insights into how environmental contaminants may influence AMR.

P2-123 Survival of *Salmonella enterica* on Dry-Inoculated Fresh Peaches During Retail and Consumer Storage Conditions

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Introduction: A recent salmonellosis outbreak in the U.S. was linked to peaches plausibly contaminated via fugitive dust from a nearby farm. This study examined the fate of *Salmonella enterica* on peaches following dry inoculation and storage in different packaging containers and at different storage conditions.
Purpose*:* To examine the survival of *S. enterica* on dry-inoculated peaches during retail and consumer storage in five different packaging containers.
Methods*:* Peaches were inoculated with *S. enterica* at 5 log CFU/peach using a dry silica carrier in a sealed box with two fans blowing at 1.3 m/s for 1 min. Peaches were transferred to plastic (produce bags with ties, perforated bags, or clamshells) or cardboard containers (bubble trays or boxes). Containers were stored at 5°C/95% RH (consumer storage) for up to 28 d or at 18 or 25°C/45% RH for up to 14 d (retail display). *S. enterica* was enumerated from peaches throughout storage. Three independent trials were conducted with triplicate samples at each timepoint. Populations were statistically compared

using ANOVA (p<0.05).

Results: The average initial population of *S. enterica* on the peaches was 4.45±0.70 log CFU/peach. After 7 d storage at 5°C, *S. enterica* was significantly higher in the bags with ties (5.37±0.87 log CFU/peach) than in the other containers; however, no population differences between the containers were observed after 28 d. At 18°C, *S. enterica* was also significantly higher in the bags with ties after 14 d (5.07±0.72 log CFU/peach) compared to the other plastic containers, but not different than the cardboard containers. During 25°C storage, populations decreased in all containers except for the bags with ties, where the population after 14 d was 4.35±0.44 log CFU/peach.

Significance: Results from this study provide information on how dry inoculated *S. enterica* survives during both retail display and consumer storage of peaches in different containers.

P2-124 Plasmid-Associated Antimicrobial Resistance, Virulence, and Metabolic Genes in Diverse *Salmonella* enterica Serovars

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❖ *Developing Scientist Entrant*

Introduction: Plasmids are readily mobilized, facilitating the acquisition and transfer of genes that can impact bacterial adaptation and survival. Previous studies have focused on characterizations of plasmids carried by a handful of *Salmonella enterica* serovars.

Purpose: This study aimed to identify and characterize plasmid genomes within diverse *Salmonella* genomes to elucidate the types of cargo carried on plasmid genomes that could confer fitness benefits to the host *Salmonella*.

Methods: A total of 288 genome assemblies of *Salmonella*, encompassing (i) *S. enterica* subsp. *enterica* (*n*=245, representing 218 distinct serovars), (ii) *S. bongori* (n=13), (iii) *S. enterica* subsp. II, IIIa, IIIb, IV, and subsp. VII (*n*=5 each) were analyzed using Platon v1.3.1 to identify plasmid genomes. The presence/absence of plasmid genomes was mapped onto a phylogenetic tree inferred with IQ-TREE from core SNPs identified with ksnp3 and visualized using iTOL. EggNOG-mapper v2 facilitated the categorization of coding sequences (CDS) into clusters of orthologous groups (COG).

Results: Among the 288 *Salmonella* isolates analyzed here, 158 had plasmid genomes, which accounted for an average of 1.80% (range: 0.03-7.06%) of the total genomic content in plasmid-containing isolates. A total of 4,052 CDSs were identified representing 37 distinct COG categories. COG categories L (replication, recombination, and repair), X [mobilome: prophages, transposons, conjugation machinery (conjugal transfer *trbJ*)], and K (transcription) had the highest number of CDSs. Additionally, several CDSs were identified with a predicted function related to (i) cellular metabolism (cobalamin biosynthesis, folate), (ii) resistance to antimicrobials (β-lactams and macrolides) and heavy metals (copper, arsenic), and (iii) virulence factors (plasmid-encoded fimbriae Pef).

Significance: Overall, these results provide important new information about the types of functional categories of CDSs carried on plasmids across the genus *Salmonella*, and an understanding of why some isolates may be associated with different hosts or infection outcomes.

P2-125 Fluoresce under Duress: Assessing Plasmid pGFPuv Stability in *Salmonella* Newport Isolates

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❖ *Developing Scientist Entrant*

Introduction: The pGFPuv plasmid has been used to confer fluorescence and ampicillin resistance to foodborne pathogens such as *Salmonella enterica*. This plasmid facilitates the labeling and tracking of cells during experiments.

Purpose: The stability of the plasmid in *Salmonella* transformants indicates reliable traceability of the strain through GFP expression and ampicillin resistance in isolates.

Methods: An onion outbreak associated *Salmonella* Newport was transformed with the pGFPuv plasmid. The transformed strain was grown in 10 ml Tryptic Soy Broth (TSB) and in 10 ml TSB with 100 µg/ml (TSBA). Plasmid stability of the cells was evaluated over 10 subcultures, with a sub-culture being performed every 24 h using the previous days culture. A starting cell concentration of approximately 5±0.5 log CFU/ml was used in the growth medium at 0 h. The population of expressing GFP was determined through enumeration on Tryptic Soy Agar (TSA) and TSA with 100 µg/ml (TSAA), Stability of the newly transformed strain was compared to a *S*. Newport (11590K, beef isolate) isolate with GFP stability.

Results: In the absence of antibiotic pressure in the growth medium (TSB) and plating medium (TSA) the *S*. Newport onion isolate showed an average reduction of 6.67±.06 log CFU/mL in GFP expressing fluorescent cells from day 1(8.35±0.34 log CFU/mL) to 9 (1.68±0.11 log CFU/mL) (p≤0.05). *S*. Newport onion isolate did not have a similar reduction in GFP expression when enumerated on TSAA from TSB (p≥0.05). In contrast, without antibiotic pressure, the enumeration of TSB grown *S.* Newport (11590K, beef isolate) on TSA indicated no loss in GFP expression with no significant differences in fluorescent colony counts over the 9-day period (p≥0.05).

Significance: The pGFPuv transformed onion isolate of *S*. Newport demonstrated stability when recovered on TSA with antibiotic and can be used to study environmental persistence and improve our understanding of onion contamination by *Salmonella*.

P2-126 Rapid Typing via Infrared Spectroscopy for Targeted Identification of *Salmonella* Serotypes Relevant to Poultry

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Introduction: *Salmonella enterica* stands as a primary cause of foodborne infections, with poultry emerging as a major contributor, specifically through the prevalence of the Enteritidis, Infantis, and Typhimurium serotypes. The identification of these serotypes holds importance for effective contamination control. Fourier-transform infrared (IR) spectroscopy, an emerging technology for *Salmonella* typing, employs machine learning to associate IR spectra with a pre-trained database improving the efficiency of serotype identification.

Purpose: Evaluate the performance of determining the serotypes of *Salmonella enterica* isolated from various human, animal, and environmental sources using IR spectroscopy.

Methods: The study scrutinized 321 *Salmonella enterica* isolates, diverse in serogroups (*S.* Infantis, *S*. Enteritidis, *S*. Typhimurium, and others), sourced from humans, poultry, equine, swine, bovine feces, and surface waters. Serotyping employed sequencing and traditional methods. With a training and validation set, each isolate underwent IR spectroscopy after ethanol and deionized water treatment. Initial analysis included HCA and dendrogram construction via PCA on 963 spectra. The training phase utilized SVM_RBF with a c-value of 3 and 23 PC analyses on 80% of spectra (n=720), followed by validation on the remaining 20% (n=180). The study aimed to create an accurate model for *Salmonella enterica* classification based on spectral characteristics, enhancing identification strategies.

Results: In the training set, the infrared spectroscopy methodology through the IR Biotyper equipment correctly identified the studied *Salmonella* serotypes. After training, the serotypes of *Salmonella* Typhimurium and *S*. Infantis were identified with 100% concordance. *S*. Enteritidis also showed 100% concordance, but in 45% of cases, a low typing percentage was obtained, indicating the need to increase the number of spectra to be studied.

Significance: Infrared spectroscopy using the IR Biotyper system is fast, cost-effective, and demonstrated 100% concordance with conventional methods for *S*. Infantis and *S*. Typhimurium. Further training will improve the accuracy with a wider range of serotypes.

P2-127 Phenotypic Analysis of Multi-Drug-Resistant and Bacteriophage-Insensitive Mutant of *Salmonella* Typhimurium

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Introduction: The researchers are working in bacteriophages to alter antibiotics; nevertheless, there is not much evidence about how phage resistance affects host phenotypes.

Purpose: To investigate the physiological characteristics of bacteriophage-insensitive *Salmonella* Typhimurium mutants (BIM), focusing on antibiotic cross-resistance, motility ability test, relative gene expression patterns, and biofilm formation.

Methods: BIM strains were generated using clinically isolated, multi-drug resistant *S.* Typhimurium wild-type strains. The antibiotic susceptibility of BIMs was conducted disk diffusion assay. The motility of parental and BIMs strains, swimming, and swarming assays were conducted. BIMs were subjected to phenotype microarray (PM) analysis of over 384 phenotypes including utilization of carbon sources and sensitivity to osmolytes and pH. qRT-PCR was conducted for genotypic analysis. Biofilm formation assay was conducted on various food contact surfaces (ss, LDPE, rubber) for 24hr to 72hr.

Results: BIM induction led to changes in antibiotic susceptibility for 11 (WT4-WT4BIM) and 6 (WT6-WT6BIM) drugs. WT4BIM and WT6BIM decreased swimming ability by 47% and 100% respectively, compared to parental strains. The swarming ability also decreased by 84% and 70%, respectively. All BIMs showed consistent up-regulation of quorum sensing genes (luxS), strongly linked to enhanced biofilm formation over 72 hours on food contact surfaces. BIM strains exhibited differential expression in bacteriophage-binding receptor genes and efflux pump system genes compared to parent strains. PM experiments revealed significant changes in carbon source utilization and osmotic effects under specific conditions.

Significance: This study reveals insights into the phenotypic and molecular diversity of bacteriophage-insensitive *S.* Typhimurium in both planktonic and mature biofilm stages.

P2-128 Isolation and Characterization of Multidrug-Resistant *Salmonella*-Specific Bacteriophages and Their Antibacterial Efficiency

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Introduction: The use of phages as biocontrol agents against multidrug-resistant strains of *Salmonella* spp. is gaining attention.

Purpose: To isolate and characterize lytic bacteriophages specific for multidrug-resistant *Salmonella* enterica serovars Typhimurium both *in vitro* and on food.

Method: Bacteriophages (STP-1, STP-2, STP-3, and STP-4) were isolated from sewage samples against *S*. Typhimurium as host strains. Characteristic analysis was conducted by determining the host range, adsorption kinetics, and one-step growth kinetics. Viability analysis was performed by assessing pH and heat stability. Morphology was confirmed using a transmission electron microscope. Subsequently, *in vitro* lysis kinetics were conducted. Chicken breasts (2 x 2 cm, 5 g) were spot-inoculated with 100 µL of *S*. Typhimurium strain cocktails, followed by dispensing 100 µL of bacteriophage lysate (MOI 10, 100, 1000).

Results: The 4 phages were classified under the *Caudoviricetes* class by morphology characterization. On host range testing, they exhibited lytic activities against *S*. Typhimurium, *S*. Enteritidis, and *S*. Thompson. In the stability test, the phages exhibited resistance to heat (above 70 °C for 1 h) and pH (strongly alkaline for 24 h). Additionally, the phages had comparable adsorption rates (approximately 80% adsorption in under 5 min). Additionally, the latent periods ranged from 30 to 50 min, with respective burst sizes of 31, 218, 197, and 218 PFU/CFU. *In vitro*, bacterial challenge demonstrated that at a multiplicity of infection (MOI) of 10, each phage consistently inhibited *S*. Typhimurium growth at 37°C for 24 h. In the food test, the phage cocktail (MOI=1,000) reduced *S*. Typhimurium in artificially contaminated chicken breast meat stored at 4°C by 0.9 and 1.2 log CFU/g after 1 and 7 days, respectively.

Significance: The results point toward a promising avenue for addressing the challenge of multidrug-resistant *S*. Typhimurium in the food industry through the use of recently discovered phages.

P2-129 Comparison of a Fully Automated Liquid Crystal-Based Immunoassay with Culture Method for Detection of *Salmonella enterica* in Various Foods

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Introduction: *Salmonella* is a commonly found foodborne pathogen in many foods, including meat, seafood, dairy, fruit/vegetable, and even processed foods. The infectious dose for this pathogen is as low as one colony forming unit (CFU). It is estimated that *Salmonella enterica* causes as many as 1.3 billion cases of disease worldwide annually. Considering the high prevalence and low infectious dose of *Salmonella*, routine testing is required to ensure the absence of such bacteria in many foods.

Purpose: Many challenges of pathogen detection in foods occur from the food matrix itself. The high background microflora and complex nature of foods post many difficulties, causing false positives or false negatives. The antimicrobial compounds in foods can also suppress the growth of bacteria during the enrichment process. Recently, commercially available biosensors for rapid detection of *Salmonella* in foods are being developed to increase sensitivity, specificity, and accuracy.

Methods: In this study, we used a fully automated liquid crystal-based immunoassay to detect *Salmonella* in twenty different food matrixes, covering meat, seafood, egg products, dairy products, fruit, vegetables, high fat foods, and low moisture foods. The results were compared to the standard culture method recommended.

Results: In this study, we used a fully automated liquid crystal-based immunoassay (LCIA) to detect *Salmonella* in over 500 artificially inoculated food samples obtained from local grocery stores. Within 18 hours, including optimized enrichment and post enrichment sample purification process, the accuracy of LCIA were 100%, 95.7%, 96.5% and 97.6% for the following food groups: fruits and vegetables, egg and dairy, meat, poultry and sea food, and low moisture foods, respectively.

Significance: Our results indicated that this assay could overcome common issues of food matrix testing, such as high fat, high sugar, and low acid. This assay has been shown to be a rapid and reliable, easy to use, sample to result assay for detection of *Salmonella* in foods.

P2-130 Prevalence of Colitose Containing O-Antigen in Rare *Salmonella* Serovars

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Introduction: Modifications in the O antigen side-chain of *Salmonella enterica* is known to enhance or reduce the virulence of *Salmonella* strains. Colitose, a component of the O side chains of a few Gram-negative bacterial pathogens is rarely observed in *Salmonella* spp. Previous studies have shown that colitose is present in the *Salmonella* Adelaide, but little has been done to examine the prevalence of this and other dideoxy sugars in rare *Salmonella* serovars.

Purpose: To conduct an analysis of *Salmonella* O-antigen gene clusters to identify serovars that contain colitose and other dideoxy sugars.

Methods: The SalFoS database (https://salfos.ibis.ulaval.ca/) containing 2954 previously sequenced *Salmonella* isolates was used in this study. Shell (bash) scripts were developed to conduct *in silico* serotyping of each genome with SISTR; identify the presence of dideoxy sugar (colitose, abequose, paratose and tyvelose) transferase genes in each genome using Blast, and full genome annotation with Prokka. Finally, an R script was developed for further analysis and to filter hits that were an exact match (e-value = 0) and over the full length of the transferase or O antigen gene cluster sequence (e.g. 13,650 bp for colitose).

Results: Twenty-eight (0.95%) of the 2954 *Salmonella* sequences analyzed contained a match to known colitose transferase genes indicating that the prevalence of this sugar is rare. All isolates (8 in total) serotyped as Adelaide contained colitose, which confirms previous findings. Colitose was not found in common foodborne outbreak associated serotypes such as Enteriditis, Typhi, and Paratyphi. In addition to Adelaide, several rare serovars contained colitose including Alachua, Duisburg, Ealing, and Monschaui, which have not been previously reported.

Significance: Our findings indicate the presence of colitose transferases in rare *Salmonella* serovars that have not been well-studied to date. The findings also confirm previous reports of the low prevalence of colitose in *Salmonella* spp.

P2-131 Surveillance of *Salmonella* Presence in the Lairage Area of a Commercial Meat Processing Facility in the United States Using Boot Swabs

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Introduction: *Salmonella* is estimated to be the most common bacterial cause of foodborne illness in the United States.

Objective: Evaluate the presence of *Salmonella* in the lairage area over a year in a beef processing plant in the Midwest.

Methods: Boot swabs were collected in the lairage area over one year (n=15 each month). Boot swabs were utilized to capture fecal material from the cattle pens. Swabs followed a "Z" pattern for their collection in the lairage area and then were placed into filtered bags; these collected samples were stored in a cooler and sent overnight to the International Center for Food Excellence (ICFIE) at Texas Tech University. Upon arrival, 100 mL of Buffered Peptone Water (BPW) was added to each sample and stomached at 230 RPM for 1 minute. Subsequently, 30 mL of the stomached samples were transferred into a 7 oz sterile bag. In each 30 mL bag, another 30 mL of BAX MP + 1 mL/L Quant Solution was poured into each bag. Samples were incubated for 24 hours at 42˚C. Following the completion of incubation, the samples underwent testing using Hygiena BAX® System Q7 for Real-Time *Salmonella* PCR assay.

Results: Chi-square test of independence was used to determine if there was significant ($p<0.05$) association between the month of the year and the presence of *Salmonella* in the lairage area. High *Salmonella* prevalence was found in April, August, September, and October, with averages of 93%, 86%, 93%, and 80% respectively. There was no *Salmonella* detected in January. The months of February, March, May, and June had less than 15% positive samples, with February being February the lowest at 6%.

Significance: *Salmonella* presence at lairage area could be used as an indicator of potential risk in the meat processing facility. Therefore, surveillance should continue year-round to ensure adequate control of the pathogen, which facilitates the development of mitigation technologies for its elimination.

P2-132 Survival of *Listeria monocytogenes* on Commercial Gourmet Mushrooms During Pre-Harvest Cultivation and Post-Harvest Storage

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Introduction: Outbreaks and recalls of Listeria monocytogenes associated with commercial gourmet mushrooms, including Enoki and King Oyster mushrooms, have been reported in recent years. Current knowledge about the survival of *L. monocytogenes* on these mushrooms remains limited.

Purpose: This study aims to characterize the survival of *L. monocytogenes* on three different types of mushrooms including Trumpet Royal (TR), Alba Clamshell (AC) and Brown Clamshell (BC) during preharvest cultivation and postharvest storage.

Methods: Mushroom caps were spot inoculated with rifampin-resistant cocktails of *L. monocytogenes* with an initial level of 8.0 and 4.0 log CFU/mushroom and air-dried for 30-min until no visible inoculum observed on inoculated area. Inoculated mushrooms were cultivated in the Mella mushroom fruiting chamber at ca. 16 °C with ca. 80% relative humidity. On the other hand, inoculated mushrooms were harvested and stored in sealed Ziploc bag at 4 °C. The survival *L. monocytogenes* was monitored at Day 0, 1, 2, 3, 5, and 7 days after inoculation by harvesting and plating the entire inoculated mushroom head onto Tryptic Soy Agar supplemented with rifampicin (TSAR). Negative samples were enriched using Difico Buffered *Listeria* enrichment broth before streaking onto Modified Oxford agar supplemented with Rifampicin.

Results: The high and low inoculated *L. monocytogenes* on growing mushrooms dropped below the limit of detection after Day 2 and 0 (2.40 Log CFU/ml). *L. monocytogenes* on harvested mushrooms survived at higher levels for longer period of time. By the end of 7-day storage, 6.10, 6.15 and 3.78 Log CFU/mushroom of *L. monocytogenes* was detected on TR, BC, and AC respectively (high-inoculation trail), whereas only TR showed *Listeria monocytogenes* positive after 7-day storage (low inoculation trails). These outcomes indicated the persistent nature of *L. monocytogenes* on mushrooms at refrigerated temperature.

Significance: This project illustrated the different survival patterns of *L. monocytogenes* on growing and harvested mushrooms, providing critical information for the development of food safety plans for mushroom industry.

P2-133 Combination Use of Power Ultrasound and Organic Acids to Reduce *Listeria monocytogenes* Populations on Peaches and Apples

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❖ ***Developing Scientist Entrant***

Introduction: Recent outbreaks and recalls associated with fruits have occurred in the U.S. due to contamination with foodborne pathogens, including *Listeria monocytogenes*. Investigating novel and efficient hurdle technologies for different fruits is necessary. This study evaluated the use of power ultrasound in combination with organic acids to reduce *L. monocytogenes* on peaches and apples, two fruits with differing surface textures.

Purpose: To assess the combined use of power ultrasound with three different organic acids to reduce *L. monocytogenes* on peaches and apples.

Methods: Fresh whole peaches and apples were spot inoculated with *L. monocytogenes* at 8 log CFU/fruit and allowed to dry for 1 h. Fruits were treated with power ultrasound at 40 kHz and 5% of citric, lactic, or malic acid for 5 min. The population of *L. monocytogenes* on the fruits was enumerated before and after each treatment combination. Three independent trials with triplicate samples were performed for each condition. Population reductions were evaluated via Student's t-test; $p<0.05$ was considered significant.

Results: After inoculation and drying for 1 h, the populations of *L. monocytogenes* on the peaches and apples were 7.53±0.64 and 7.46±0.65 log CFU/fruit, respectively. No significant reduction was observed on the peaches with any of the treatment conditions. No significant reduction was also observed on the apples which were treated with citric acid. However, the *L. monocytogenes* population was significantly reduced by 1.25 and 2.03 log CFU/fruit on apples treated with lactic and malic acids with the combination of power ultrasound.

Significance: The results of this provide information on the reduction of *L. monocytogenes* on two types of fruits treated with a combination of power ultrasound and organic acids. Results suggest that fruit surface texture may play a role in the effectiveness of this treatment.

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P2-134 *Listeria monocytogenes* Ability to Persist in High and Low Concentrations of Second Generation QAC, Ster-BAC in Water and Recovery of Small Colony Morphotypes

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Introduction: The purpose of this study was to investigate the ability of two *Listeria monocytogenes* strains, Bug600 (serotype 1/2a) and ScottA (serotype 4b) to persist in lethal and sublethal concentrations of second generation QAC, Ster-BAC, and if these strains would regrow when conditions had improved.
Methods: A high and low concentrations of Ster-BAC in a 96 well plate were exposed to *L. monocytogenes* strains for 1 h kill step in water and then the real-time growth rate (by $OD_{600\ nm}$) of surviving *L. monocytogenes* cells were examined by mimicking the high and low nutrient conditions that it may encounter in some food processing environments. In another experiment, L. monocytogenes cells were exposed to a lethal Ster-BAC for 1 h kill step and then after diluting Ster-BAC, the persisting cells were allowed to grow in high and low nutrient conditions to recover if any colony morphotypes are formed. All experiments were repeated thrice, and logs transformed counts were analyzed using One way ANOVA in a completely randomized block design and means were separated by Fisher's protected LSD when $p<0.05$.
Results: The MIC of Ster-BAC was 1 µg/ml for Bug600 and 2 µg/ml for ScottA. After a rapid reduction of 5-7 logs CFU/ml when exposed to 6 µg/ml Ster-BAC in water for 1 h, a few cells of *L. monocytogenes* were still recoverable for one of the two strains (ScottA) in water. When such persisting cells were incubated in high or low nutrition conditions for 24 h, they gave rise to 1-2 CFU/ml of *L. monocytogenes* progenies (p <0.05). Some survivors of both strains displayed a unique phenotype in which colony size varied widely irrespective of any concentration or any nutrition level tested.
Significance: Our results show that *L. monocytogenes* strains can persist in some concentrations of second generation QACs. Small colony morphotype variants have been isolated which might be an adaptative mechanism.

P2-135 Association of Accessory Gene Loci with Environmental Stress Tolerance in Listeria monocytogenes

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❖ ***Developing Scientist Entrant***

Introduction: *L. monocytogenes* strains present various robust stress adaptive capabilities, in part due to genetic differences among strains, and these genetic variations can contribute to various *L. monocytogenes*' ability to grow under diverse environmental conditions.
Purpose: To quantify the association of specific accessory gene loci with growth parameters of *L. monocytogenes* strains under various environmental stresses.
Methods: A total of 317 *L. monocytogenes* strains, representing 4 lineages and 80 sublineages, were subjected to growth in BHI broth under multiple stress conditions (varying pH, salt concentrations, and temperatures), and OD600 monitored over time in triplicate. The gcplyr R package was employed to fit growth parameters, and Roary was used to identify core and accessory genes. TreeWAS implemented in R was applied to identify accessory genes significantly associated with growth capabilities under each of the conditions.
Results: A number of accessory genes were significantly associated with stress-condition growth parameters. Five loci were significantly ($p<0.05$) associated with growth at pH 8.5, while 9 loci were significantly associated with pH 5.5 adaptation, including virulence factor *inlJ*. Under salt stress, 16 loci were significantly associated with growth in 7% NaCl, and 7 with growth in 4% NaCl. At 42°C and 24°C, 12 and 17 loci were significantly linked, respectively. Some genes such as *inlJ*, *lmo2131*, a regulatory subunit of cAMP-dependent protein kinases which has signal transduction function, and *lmo2303*, comK associated prophage that contributes to intracellular growth, were repeatedly presented as significant loci under various stress conditions.
Significance: Identifying these loci provides a deeper understanding of the genetic factors contributing to the resilience of L. monocytogenes in varying environments. This knowledge can enhance predictive models for its growth in complex conditions, such as food processing environments, and may explain the prevalence of certain subtypes in specific niches.

P2-136 Functional Analysis of llsX, for the Listeriolysin S Production in *Listeria monocytogenes*

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Introduction: Listeriolysin S (LLS) serves as a virulence factor, impacting the intestinal microbiota through antimicrobial activities to facilitate the survival of *Listeria monocytogenes* in gut. The LIPI-3 operon, encompassing eight genes (llsABDGHPXY), includes the llsX gene, the function of which remains unknown.
Purpose: This study aimed to elucidate the role of the *llsX* gene in the production and secretion of LLS.
Methods: A mutant strain F2365△*llsX* was constructed using a temperature-sensitive suicide vector, pHoss1 through a double allele crossover exchange. Complete deletion was confirmed with sequencing, and the expression of *llsX* and other genes in LIPI-3 was confirmed with RT-PCR. Functional changes by the deletion were confirmed in broth medium, Caco-2 cells and under fecal fermentation condition. Toxicity and virulence were assessed through lactate dehydrogenase (LDH) and adhesion assay on Caco-2 cells.
Results: The expression of *lls* genes and the survival of *L. monocytogenes* F2365 WT and F2365△*llsX* were evaluated under co-culture conditions with lactic acid bacteria, gut microbiota via fecal fermentation, and the metabolites of gut microbiota. Although the expression of the *lls* genes and the secretion of LLS was observed, the mutant strain showed reduced growth under the co-culture conditions. Cytotoxicity and adhesion assays revealed no significant differences between F2365 WT and F2365△*llsX* by LDH assay with 21.8 and 24.3%, and adhesion of 1.68% and 1.71%, respectively. The *llsX* gene did not have a direct effect on *L. monocytogenes* infection *in vitro* model.
Significance: This study is the first to uncover the function of llsX, offering novel insights into LLS production. Additionally, it provides information on the mechanisms involved in modulating gut microbiota and enhancing survival under food processing conditions, thereby conferring a competitive advantage.

P2-137 Comparative Genomics and Phenotypes of *Listeria monocytogenes* Isolated from Enoki Mushrooms in South Korea and China

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Introduction: Recent recalls and outbreaks of *Listeria monocytogenes* contamination in exported enoki mushrooms from Korea and China have raised concerns regarding persistent contamination.
Purpose: This study aimed to isolate *L. monocytogenes* from Korean and Chinese enoki mushrooms and subsequently characterize and compare their genomes and phenotypes.
Methods: *L. monocytogenes* strains were isolated from Korean (n=56) and Chinese (n=84) enoki mushrooms. The serotype of isolates was confirmed. Biofilm formation was tested with crystal violet assay. Antibiotic-resistant profile of the isolates against 11 antibiotics was determined using a disc diffusion assay. Whole-genome sequencing was performed on the isolates, and multi-locus sequence typing (MLST), core genome MLST, and clonal complex analysis were conducted on the isolated strains from Korea (FSCNUKL) and China (FSCNUCL).
Results: *L. monocytogenes* was identified in enoki mushrooms with detection rates of approximately 17.8%（10/52）from Korea and 15.5%（13/84）from China. FSCNUKL and FSCNUCL biofilm formation were not significantly different by 0.29±0.11 and 0.34±0.12, respectively. Multi-resistance to lincomycin, ampicillin, penicillin G, and bacitracin was observed in 40% of isolates. Major virulence and antibiotic-resistance genes were present among the isolates. The serotypes of FSCNUKL were 1/2a and 1/2c, while FSCNUCL was detected with only the 1/2a serotype. The isolates belonged to sequence types

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of ST5, ST7, ST8 and ST9.

Significance: Continuous monitoring is required due to the persistent isolation of *L. monocytogenes* in Korean and Chinese enoki mushrooms. This study contributes to understanding the characteristics and genetic differences of the *L. monocytogenes* from enoki mushrooms. The findings serve as valuable references for detection and prevention measures.

P2-138 Different *Listeria monocytogenes* Lineages Occupy Unique Ecological Niches in the Soil

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Introduction: *Listeria monocytogenes* consists of at least 4 evolutionary lineages (I, II, III, and IV) and may transmit from soil to foods, subsequently causing host infection, but our understanding of the ecology of these lineages in soil remains largely unknown at a large spatial scale.

Purpose: The purpose of this study is to bridge the knowledge gap in the ecology of *L. monocytogenes* in the soil at a nationwide scale and assess the risk of transmission from soil to humans.

Methods: We analyzed whole genome sequencing (WGS) data of 177 *L. monocytogenes* strains representing lineage I, II, and III that we isolated from 1,004 soil samples from the United States. Correlation analysis was conducted to assess the relationship between the presence of *L. monocytogenes* lineages and environmental variables capturing geolocation, soil property, climate, and land use. WGS data of soil strains of *L. monocytogenes* lineages was further compared with WGS data of clinical strains available in the Pathogen Detection database.

Results: We found that lineage III is the most prevalent *L. monocytogenes* lineage in soil followed by lineage II and lineage I. The habitats of these three lineages rarely overlap and exhibit significantly different abiotic and biotic environmental conditions. We showed that even though *L. monocytogenes* lineage I is not prevalent in soil, it exhibits a much higher risk of transmission from natural environments to humans compared to the other two lineages. This higher risk may be related to the lower degree of dispersal limitation that we observed in this lineage.

Significance: Our study reveals substantial differences in the ecological niches and potential risk of transmission to humans among *L. monocytogenes* lineages in the soil environment, necessitating the design of lineage-specific biocontrol tools.

P2-139 *Listeria monocytogenes* and Other *Listeria* spp. on Food Contact Surfaces of Canadian Ready-to-Eat Red Meat and Poultry Product Establishments

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Introduction: Under Canada's National Microbiological Monitoring Program (NMMP), Canadian ready-to-eat (RTE) red meat and poultry product establishments are monitored for the presence of *Listeria monocytogenes* and other *Listeria* spp. to assess compliance of these establishments with Canada's Policy on *Listeria monocytogenes* in ready-to-eat foods (2023).

Purpose: Results from RTE red meat and poultry establishment environmental samples collected under the NMMP between April 1, 2018 and March 31, 2023 were summarized to compare the prevalence of *L. monocytogenes* and other *Listeria* spp.in these establishments.

Methods: Between 5 and 10 food contact surfaces per establishment were swabbed during food production. Individual swabs were combined to create composite swab samples, which were screened for the presence of *Listeria* spp. The presence of *L. monocytogenes* and other *Listeria* spp. in positive screening samples was confirmed by a culture-based method. All methods were from Canada's Compendium of Analytical Methods.

Results: A total of 4797 composite swab samples were collected from 276 establishments. *Listeria* spp. were detected in 130 (2.71 %) of the samples, 47 (0.98%) of which were confirmed as *L. monocytogenes*.

Significance: Canada's Policy on *Listeria monocytogenes* in ready-to-eat foods (2023) recommends that food producers perform environmental testing of food contact surfaces in ready-to-eat (RTE) food production establishments for *Listeria* spp. to assess the effectiveness of their manufacturing practices in controlling *L. monocytogenes* in these environments. The results presented here underline the importance and value of environmental sampling to monitor and detect *Listeria* spp. as a suitable indicator of the potential loss of control within the production environment.

P2-140 Comparative Genomics of Extra-Intestinal Pathogenic *E. coli* from Human Clinical and Food Samples

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Introduction: Extraintestinal pathogenic *Escherichia coli* (ExPEC) causes a range of serious human diseases and complications, however, understanding of the sources of ExPEC is poor.

Purpose: Comparative genomic analyses of ExPEC from human clinical and food (poultry) samples to assess the risk of poultry as a potential reservoir of high risk ExPEC.

Methods: Forty human clinical and 43 poultry (chicken or turkey meat) isolates were confirmed to be ExPEC by multiplex PCR. Genomes were sequenced using long- or short-read technologies. Long read sequences were assembled using Flye 2.9.2. Assembly of short read sequences and characterization of all genome assemblies were performed using the GEAbash bioinformatic pipeline. Prevalence was compared using Fisher's exact test.

Results: Overall whole genome sequencing of ExPEC strains identified 101 virulence genes, 7 phylogroups, 36 sequence types (ST), and 32 antibiotic resistance genes (ARGs). Most of the virulence genes (70/101) were detected in both human and poultry isolates, with some virulence factors unique to each sample source. The majority of the human isolates (34/40) and poultry isolates (12/43) belonged to phylogroup B2 known for better persistence in the human gut and high virulence. Another predominant phylogroup among poultry isolates was phylogroup F (10/43), a group associated with devastating poultry diseases and potential zoonotic risk. Highly virulent ST95 was represented among strains from both sources. Prevalence of ARGs were significantly higher in poultry (31/43) as compared to human isolates (18/40). Resistance against nine different classes of antibiotics was identified in both poultry and human isolates.

Significance: Distribution of poultry isolates across highly virulent phylogroups and STs, together with abundance of virulence factors and ARGs suggest that poultry may act as a source of high risk ExPEC leading to serious human diseases.

P2-141 Modulation of the Virulence of Multidrug-Resistant *E. coli* O104:H4 by Subinhibitory Concentrations of Ampicillin

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✦ ***Undergraduate Student Award Entrant***

Introduction: There is concern about the effect of antibiotics on the physiology of β-lactamase-producing bacteria, such as *E. coli* serotype O104:H4. An interesting question is whether the virulence of bacteria is altered by exposure to subinhibitory concentrations of antibiotics to which bacteria are resistant.

***Purpose*:** To determine the effect of subinhibitory concentrations of ampicillin (inducer of β-lactamases) on virulence factors of *E. coli* O104:H4.

***Methods*:** Sensitized inoculum was prepared by growing bacteria in LB media with 0.1, 0.3, or 0.5 mg/ml of ampicillin (that not affected viability of

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bacteria) for 4 h at 37ºC. This inoculum was used for assays of transformation capacity with exogenous DNA (plasmids and PCR-amplified DNA sequences), swarming motility, biofilm formation, curli production, Shiga toxin production, and expression of related virulence genes.

Results: Ampicillin at the concentrations used, increased the transformation ability, detecting the highest number of transformants (>10^4 CFU/ng DNA; $p \leq 0.05$) after exposure to DNA sequences of resistance to spectinomycin, altering the expression of genes related to homolog recombination (*recA* and *lexA*). In addition, bacteria pre-treated with 0.5 mg/ml of ampicillin and then exposed to 0.1 and 0.3 mg/ml of antibiotic during the swarming and biofilm assays, exhibited higher swarming mobility (up to 7.6 cm, vs 6.0 cm of control; $p \leq 0.05$) and biofilm production (up to 1.9-fold; $p \leq 0.05$ compared to control). Also, significant overexpression of the genes *flhC* (≤16.1-fold), *fliA* (≤22.1-fold), *csgA* (≤3.6-fold), *csgD* (≤9.1-fold), *stx2a* (≤32.2-fold) and *blaTEM-1* (≤5.5-fold) was observed, showing positive correlation in most of cases.

Significance: Ampicillin-resistant E. coli O104:H4 increased the expression of its virulence factors when exposed to most of the subinhibitory concentrations of ampicillin analyzed. This information should be considered for therapeutic measures against this foodborne pathogen and recommends studying the potential risks of antibiotic residues in food or environmental samples on the pathogen.

P2-142 NF Validation Study of a Chromogenic Agar Method for Enumeration of *E. coli* and Coliforms in Animal Feed

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Introduction: RAPID'*E.coli* 2 is an ISO 16140-2 validated method based on chromogenic substrate by NF Validation for the enumeration of *E. coli* and other coliforms in human food and environmental samples. The principle of the medium relies on the simultaneous detection of 2 enzymatic activities, β-D-Glucuronidase (GLUC) leading to pink coloration and β-D-Galactosidase (GAL) leading to a blue coloration. The enumeration is obtained by colony counting of the blue (coliforms) and violet (*E. coli*) colonies after 21 ± 3hr of incubation at 37°C.

Purpose: The purpose of this study was to extend the validation of the test method to include the animal feed category according to the ISO 16140-2:2016 validation protocol.

Method: The candidate method was compared to the ISO 4832:2006 standard (coliforms) at 30°C and 37°C incubation temperature, and the ISO 16649-2:2001 (*E. coli*) standard at 44°C. Three types of environmental samples were tested: 1/ pet food, 2/ animal feed including cereals and flour, 3/ ingredients for animal feed. Naturally and artificially contaminated samples were analyzed. The relative trueness and the accuracy profile were evaluated.

Results: On the new category evaluated, the bias between the reference methods and the test method varied from 0.06 log CFU/g (coliforms at 30°C and 37°C) to 0.07 log CFU/g (*E. coli*). The standard deviation difference for all categories varies from 0.14 log CFU/g (coliforms at 30°C) to 0.16 log CFU/g (coliforms at 37°C and *E. coli*). The accuracy profiles fall into the acceptability limits (+/- 0.5 log).

Significance: The chromogenic method enabled an accurate enumeration of *E. coli* and coliforms in environmental samples. The result is delivered in only 18 hr without confirmation step on a single plate for two targets.

P2-143 The Impact of Hypo-Osmotic Stress on Heat Resistance in Wastewater-Borne ExPEC Strains

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❖ ***Developing Scientist Entrant***

Introduction: Certain strains of *Escherichia coli*, and in particular the extraintestinal pathogenic *E. coli* (ExPEC), appear to be remarkably adapted to survive waste treatment, potentially representing an important public health threat due to their ability to withstand sanitary treatment processes (e.g., wastewater treatment).

Purpose: This study investigated heat resistance of wastewater-derived ExPEC in order to better understand the underlying mechanisms that allow this pathogen to survive sewage treatment.

Methods: We examined heat resistance in various ExPEC strains isolated from chlorinated sewage or treated wastewater effluents. Strains were exposed to iso-osmotic (PBS) or hypo-osmotic conditions (deionized water) under short-term (~ 30 sec) and long-term exposure (24 h), and their heat resistance was assessed under a range of temperatures (25ºC to 66ºC) and for varying lengths of time (0 – 60 minutes). A modified most probable number (MPN) spot assay was used to enumerate surviving bacterial cells. One or two-way analysis of variance (ANOVA) with Bonferroni's post-test, was conducted to evaluate the significance of hypo-osmotic stress on heat resistance.

Results: ExPEC strains exhibited a significant and rapid increase in heat resistance when exposed to deionized water, with some strains displaying up to a 1000-fold increase in resistance compared to iso-osmotic conditions in PBS. The induction of resistance was rapid (i.e., within seconds) and reversible, suggesting an anticipatory proteomic regulation of this heat-resistant phenotype. Conversely, a reference lab strain (ATCC 25922) and a reference ExPEC strain (CFT073), both demonstrated substantial declines in culturability under the same heat shock regimes.

Significance: This study demonstrates that water (extreme hypo-osmolarity) appears to act as an anticipatory trigger of a heat-resistant phenotype in wastewater-derived ExPEC. This novel finding may explain why ExPEC survive the sewage treatment environment. Understanding the mechanisms may guide the development of more effective treatment strategies to mitigate the risks posed by such pathogenic bacterial strains.

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P2-144 Prevalence and Antimicrobial Resistance of *E. coli* and *Enterococcus* from Retail Pork in Hawaii

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Introduction: Antimicrobial resistance (AMR) is a significant health concern affecting people worldwide. Both the AMR genotype and phenotype of bacteria isolates from food products can be determined to assess the risk of AMR in retail foods to humans. The U.S Food and Drug Administration (FDA) National Antimicrobial Resistance Monitoring System (NARMS) tracks AMR trends in retail foods.

Purpose: To expand NARMS efforts in Hawaii by assessing the AMR trends among *Escherichia coli* (*E. coli)* and *Enterococcus* isolates from retail pork samples.

Methods: Retail pork samples were collected monthly from randomly selected grocery stores in Hawaii in 2020 and 2022. The NARMS Retail Meat Isolation Protocol was utilized to test *E. coli* and *Enterococcus* from the samples. AMR phenotype for *Enterococcus* isolates was assessed by the FDA Center for Veterinary Medicine (CVM) using antimicrobial susceptibility tests (AST). *E. coli* isolates underwent whole genome sequencing (WGS) to identify antimicrobial resistance genes.

Results: A total of 141 samples were tested throughout the sampling period. Among tested samples, the culture positive rate of *E. coli* was 26.9% while that of *Enterococcus* was 71.6%. The WGS identified five distinct AMR genotypes among *E. coli* isolates from 2020, with all isolates carrying the gene associated with a multidrug efflux pump, *mdf* (*A*). Of the *E. coli* isolates, 71.4% carried *mdf* (*A*) alone, while the remaining four AMR genotypes carried *mdf(A)* and one or more additional AMR genes. The AST for *Enterococcus* revealed that 11.8% of isolates were resistant to chloramphenicol, 11.8% were resistant to streptomycin, 17.6% were resistant to gentamicin, 64.7% were resistant to tetracycline, and 100% were resistant to quinupristin/dalfopristin. All isolates were susceptible to the other tested drugs.

Significance: Monitoring the prevalence and AMR genotype and phenotype among *E. coli* and *Enterococcus* in pork sold in Hawaii can increase the resolution of AMR surveillance.

P2-145 Validation of the 3M™ Molecular Detection System for Detection of Shiga Toxin-Producing *E. coli* (STEC) from Environmental Swabs

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC), including *E. coli* O157:H7 and related strains, pose a considerable threat as a foodborne pathogen due to its capacity to induce severe health repercussions and its pervasive presence. The landscape of foodborne pathogen detection has been substantially altered by nucleic acid amplification technologies, offering not only faster outcomes but enhanced specificity in contrast to conventional culture-based approaches. The 3M Molecular Detection System utilizes a technique based on real-time loop-mediated isothermal amplification (LAMP) for gene amplification, contributing to efficient and accurate pathogen detection.

Purpose: The primary goal of this research is to ascertain the efficacy of the molecular-based detection system in accurately identifying STEC and *E. coli* when subjected to co-culture conditions. Understanding the system's reliability in such conditions is crucial for its application in real-world scenarios.

Methods: We compared the performance of a commercial molecular detection methodology for identifying STEC to the procedure outlined in the USDA FSIS Microbiology Laboratory Guidebook (MLG) 5C.03 under co-culture conditions. Molecular detections were performed at 8, 14, and 24-hour intervals using the MDS to assess performance over time.

Results: The molecular detection method exhibited 100% accuracy and sensitivity for *E.coli* and STEC detection after 24 hours of enrichment, similar to the results obtained with the reference method. On the other hand, the molecular method demonstrated reliable performance by detecting positive samples at earlier enrichment stages (8 and 14 hours), identifying between 40% to 80% of the total positive samples.

Significance: This study indicates that the molecular detection system is a reliable and accurate method for detecting *E. coli* and STEC, even at early stages of enrichment. It offers a viable alternative to more time-consuming detection methods. By minimizing detection time, this methodology enhances response times in cases of contamination on the food production line, thereby improving food safety and contributing to public health.

P2-146 Resistance to Critically Important Antimicrobial Drugs in *Escherichia coli* Isolated from Three Food Animals (Catfish, Cattle and Goat) in Alabama

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Introduction: Antimicrobials are rarely used to treat intestinal infections caused by Shigatoxigenic *Escherichia coli* (STEC) like O157:H7 due to hemolytic uremic syndrome (HUS) risk.

Purpose: This study investigated resistance to generic antimicrobial drugs in Escherichia coli (E. coli) in catfish, cattle, and goats in Alabama.

Methods: Here, catfish were recovered from fishponds (n=15) in Lee County, and fecal samples were obtained from cattle (n=15) and goats (n=15) at the Tuskegee University farms. These samples were cultured on MacConkey agar, and the isolates were confirmed phenotypically and genotypically. After that, the Kirby-Bauer test by antibiotic discs (n=8) was performed.

Results: Upon analysis, we isolated *E. coli* from all 45 samples. Fish isolates showed the most resistance trend to at least one antimicrobial (7/8), followed by cattle isolates (3/8) and then goat isolates (2/8). The cattle isolates showed resistance to vancomycin (93.3%), goat isolates showed resistance to vancomycin and linezolid (93.3% each), and fish isolates showed resistance to azithromycin and linezolid (93.3% each), cefepime (80%), aztreonam and vancomycin (73.3%) and trimethoprim-sulfamethoxazole (53.3%). All the other isolates showed a resistance of lower than 50%. All eight antimicrobials were critically important antimicrobials (CIAs), defined as drugs from an antimicrobial class that are the sole or one of the limited available therapies used to treat severe bacterial infections in humans. The preliminary results of this small number of isolates provide the basis for a broader study investigating AMR occurrence in these three species within Alabama.

Significance: The occurrence of these antimicrobials in catfish is worrying since none of the antimicrobials tested is approved for use in catfish. Overall, we recorded antimicrobial resistance (AMR) to critically important antimicrobials (CIA) in these three food-producing animals, which may be a public health concern.

P2-147 Metagenomic Approach to Investigating the Efficacy of Probiotics for *Salmonella* Infantis Reduction in Broiler Chickens

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Introduction: Probiotics have gained increased usage as an alternative to antibiotics for growth promotion. However, it is not clear if they are effective and safe for food-borne pathogen control.

Purpose: To gain insights into the efficacy of probiotics for *Salmonella* reduction in broiler chickens and their impact on the reservoir of antimicrobial resistance genes.

Methods: Five hundred and twenty 1-day-old Cobb 500 broiler chicks (260 males and 260 females) were randomly assigned to four treatment groups: (T1) Commercial probiotic (added to feed), (T2) ARS probiotic (applied to litter), (T3) No probiotic (*Salmonella* positive control), and (T4) No probiotic (*Salmonella* negative control). All chicks were raised on floor pens (4 – 6 pens/treatment) and each pen held twenty-six birds (13 males and 13 females). At day zero, six birds from each pen in treatment groups 1, 2, and 3 were gavaged with a cocktail of antibiotic susceptible and multidrug resistant strain of *Salmonella* Infantis. At 7 days post-infection, six birds from each treatment group were euthanized and their ceca was collected and used for Shotgun metagenome sequencing.

Results: Broilers that received probiotics (T1 and T2) harbored significantly ($p<0.05$) lower abundance of *Salmonella* than positive control. Members of the family Enterobacteriaceae represented 1.39±0.78% of the total cecal microbiome for each treatment group, however, within Enterobacteriaceae, the relative abundance of *Salmonella* was 15.13±22.12%, 11.20±8.02% and 31.28±22.25% for T1, T2, and T3, respectively. The abundance of *Salmonella* in broilers from T4 was below the detection limit of our metagenome sequencing effort. There was no significant difference in the abundance of antimicrobial resistance genes between treatments ($p>0.05$) except for two aminoglycoside resistance genes (*aad(6)* and *aph(3')-IIIa*) that were higher in birds from T2 compared to T3 ($p=0.03$).

Significance: This study showed that probiotics have the potential to reduce *Salmonella* levels in the cecal microbiome of chickens with minimal impact on the reservoir of antimicrobial resistance genes.

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P2-148 Gas Phase Hydroxyl-Radical Process for Reducing *Salmonella* and *Campylobacter jejuni* on Inoculated and Naturally Contaminated Raw Poultry Parts with No Change in Quality Metrics but Increased Shelf Life

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Introduction: The performance standards for *Salmonella* (<15.4%) and *Campylobacter* (<7.7%) carriage on raw poultry parts are becoming increasingly stringent. Consequently, there is a need for interventions to meet the performance criteria without negatively affecting meat quality.

Purpose: To validate and verify a gas phase-hydroxyl radical process to reduce *Salmonella* and *C. jejuni* carriage of raw poultry parts with no negative effect on the visual, storage and cooking quality.

Methods: A *Salmonella* cocktail and five strain cocktail of *C. jejuni* were spot inoculated onto raw chicken wings (7 log CFU/wing). Hydroxyl-radicals were formed via UV-C (254 nm) degradation of hydrogen peroxide vapor and ozone gas. The chicken parts were passed through the reactor and survivors recovered by the rinse method. Verification trials were performed on non-inoculated parts that were passed through the optimized hydroxyl-radical reactor with survivors being detected using enrichment followed by RT-PCR. Color, firmness, drip loss and cook loss were selected as quality metrics. Shelf-life trials were undertaken at 4°C with indicator counts and visual appearance being assessed over 12 days.

Results: The hydroxyl-radical process was optimized by determining the impact of hydrogen peroxide concentration (1-6%) and UV-C dose (15 – 546 mJ/cm2) as independent variables. Log reduction of Salmonella and color change representing the dependent variables. The optimized treatment (1% H_2O_2, 32 mJ/cm^2, ozone 12 mg) supported a 2.08±0.13 and 1.73±0.20 Log CFU reduction of *Salmonella* and *C. jejuni* respectively. The treatment reduced the prevalence of *Salmonella* and *Campylobacter* on non-inoculated poultry by 6% and 4% respectively. There was no significant change in quality metrics but an increase in shelf-life by two days.

Significance: Gas phase hydroxyl radical process can be applied as part of a hurdle approach to reduce the carriage of *Salmonella* and *C. jejuni* on poultry parts with no loss of quality while extending shelf-life by two days.

P2-149 Cultivated Meat Production: Microbial Contamination Trends and Mitigation of *Staphylococcus aureus* Contamination with Antimicrobial Peptide 1018-K6

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❖ ***Developing Scientist Entrant***

Introduction: As the cultivated meat and seafood industry progresses towards commercial production, the potential impact of microbial contamination in such products needs to be quantified alongside the evaluation of effective antimicrobial agents.

Purpose: This study investigated microbial contamination possibilities in cultivated meat production and evaluated the efficacy of antimicrobial peptides (AMP), particularly 1018-K6, as a substitute for traditional antibiotics in reducing microbial contamination.

Methods: GFI APAC conducted a survey with 17 cultivated meat product producers regarding their microbiological contamination prevention and testing practices. We also evaluated the growth potentials of 12 bacterial strains isolated from cell culture environments and human hands in C2C12 myoblast cell culture. The antimicrobial activity of AMP 1018-K6 was tested against the selected bacteria accordingly.

Results: The majority of respondents identified process contamination as the primary source of microbial contamination. The batch contamination rate varied from 0% to 52%, averaging at 11.2% (n=12), with bacteria identified as the primary contaminant (n=11). Only a minority of respondents had a HACCP plan in place at the time of the survey (n=7), under half (n=8) were conducting microbial testing of their facilities. In C2C12 culture, *Staphylococcus aureus* from human hands showed the highest growth potential compared to other isolates ($p < 0.05$) and thus was selected as the focus of the mitigation study. In the mitigation study, AMP 1018-K6 demonstrated prominent antimicrobial activity against *S. aureus* with 20 µmol/L reducing the bacteria to below detectable limits.

Significance: This study demonstrates that microbial contamination, particularly bacterial, currently presents a challenge as cultivated meat companies scale up their bioprocess toward commercial food manufacturing. A majority were yet to integrate standard food industry contamination protocols that could reduce contamination rates. Our results then highlight the potential of AMP 1018-K6 as an effective antimicrobial agent which companies could employ as a mitigation strategy.

P2-150 Analysis of the Performance of Disinfection Treatments in the Reduction of Salmonella Contamination in Costa Rican Fresh Chicken

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Introduction: Although it is well known that Salmonella is part of chicken microbiota, its presence in poultry meat commercialized in Costa Rican markets is poorly explored. It is mostly assumed that the prevalence and resistance patterns for Salmonella are similar to the ones observed in published literature.

Purpose: The contamination levels of *Salmonella* in 98 pieces of raw chicken obtained from Costa Rican markets were investigated using the USDA methodology. The isolates were tested for antibiotic resistance by disc diffusion method; disinfectant resistance against chlorine (50 ppm), peracetic acid (250 ppm), and lactic acid (6 %) was also tested by exposing *Salmonella* to these compounds for 20 minutes at 4 °C; in tube assay and in the chicken. The concentrations for the disinfectants were established by considering the current regulations and the minimal inhibitory concentration observed in previous *in vitro* experiments.

Methods: More than two-thirds of the analyzed pieces were contaminated with *Salmonella* (69/98), and 21 % of the pieces showed contamination levels higher than 10 CFU per gram of meat. Also, more than half of the isolates were resistant to streptomycin (62 %), tetracycline (93 %), ampicillin (55 %), and chloramphenicol (74 %).

Results*: Salmonella* reductions of (0.05±0.01) log/ml were observed in tube when using lactic acid, peracetic acid, or chlorine, each alone or in combination; the exception was the combination of peracetic acid and chlorine, which reduced more than 5 log *Salmonella*. Nevertheless, when tested in the raw chicken, this combination reduced just (0.36±0.07) log/g of the inoculated *Salmonella*.

Significance: To our knowledge, this is the first study that quantifies Salmonella in fresh chicken from Costa Rica. These findings show the necessity of corrective actions to improve disinfection in the poultry industry, and the data can be used to further understand the salmonellosis risk for Costa Rican consumers.

P2-151 Exploring Peroxyacetic Acid and Chlorine as Promising Antimicrobial Agents in Intermittent Spray Chilling for Enhancing Pork Safety

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Introduction: Meat industry faces significant food safety challenges from pathogens like Shiga toxin-producing *Escherichia coli* (STEC). Some previous studies suggest that STEC might be more prone to oxidative damage at low temperature.

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Purpose: This study aimed to evaluate the effectiveness of intermittent spraying of peroxyacetic acid and chlorine solution during the simulated carcass chilling process to inactivate *Escherichia coli* on pork skin surface.

Methods: The samples were inoculated with a five strains cocktail of *E. coli* at 6.11 log CFU/cm^2. The spray treatment involved a hollow cone nozzle operating at 40 psi, 34.04 ml/s at 15 cm for 12 cycles of 4s at 30 min intervals (n=9). This was followed by 18 hours of chilling at 0-2°C in a custom-built temperature-controlled cabinet.

Results: The *E. coli* counts in dry chill samples decreased by 0.45 Log CFU/cm^2 (p>0.05). Intermittent spraying of PAA or chlorine significantly reduced *E. coli* counts in a concentration-dependent manner. PAA at 200 and 400 ppm and chlorine at 50 and 200 ppm reduced *E. coli* counts (p<0.05) by 0.94 and 1.85 log CFU/cm^2, and 0.63 and 1.07 log CFU/cm^2, respectively. Subsequently, 400 ppm PAA was applied at 8.04, 15.52, and 22.21 ml/sample, using a full cone nozzle at 40 psi and a 30 cm distance with a flow rate of 3.59 ml/s for 12 cycles. Increasing the volume did not always significantly affect *E. coli* reduction, with observed reductions of 1.46, 1.78, and 1.81 log CFU/cm^2, respectively.

Significance: This study concludes that applying PAA or chlorine solutions during spray chilling as antimicrobial interventions could effectively control *E. coli* contamination on pork. However, optimizing spraying parameters, including volume, frequency, time, distance, and nozzle type, is essential to achieve higher efficacy and lower chemical usage.

P2-152 Eugenol Nanoemulsion: A Natural Antimicrobial for Inactivating *Salmonella* Enteritidis on Broiler Chicken Skin for Improving Product Safety

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❖ ***Developing Scientist Entrant***

Introduction: Contamination of poultry meat with *Salmonella* Enteritidis (SE) has been an ongoing challenge that requires the development of novel antimicrobial wash strategies. Eugenol (EG; phytocompound from clove) has been shown to exert anti-SE efficacy, however, the low water solubility of EG hampers its application as a carcass wash treatment.

Purpose: This study investigated the efficacy of Eugenol in its nanoemulsion form (EGNE) as dip treatments in inactivating SE on chicken skin.

Methods: EGNE was formulated with Gum Arabic and lecithin (GAL) as emulsifiers. Chicken skins were spot-inoculated with a 5-strain cocktail of SE (10^7 CFU/sample), followed by dipping in water (control) or water containing Peracetic acid (PAA) 0.02%, GAL 0.5%, EG, or EGNE at 0.3, 0.6, 1.25% for 15, 30, or 240 min at 4°C. Post treatment, the surviving SE on chicken skin and in wash water was enumerated. All experiments had triplicate samples, repeated thrice, and analyzed using one-way ANOVA (p<0.05).

Results: EGNE had a particle size of 82 nm, PDI of <0.3 and zeta-potential of ~ -36.70 mV. In baseline (SE inoculated skin, not washed), ~7.27 log CFU/sample SE were recovered. Washing with water or GAL did not reduce SE on skin. All nanoemulsion concentrations were effective, as early as 15 min, in reducing SE by ~0.81, 1.45, 1.74 log CFU/sample, respectively, as compared to control. EGNE 1.25% was more effective than corresponding EG treatment at all timepoints and reduced SE by ~3.06 log CFU/sample as compared to control by 240 min. No significant difference between EGNE 1.25% and PAA was observed at 15 or 30 min wash time. However, at 240 min wash time, EGNE 1.25% was more effective than PAA and reduced SE by additional 1.5 log CFU.

Significance: EGNE could potentially be used as a natural antimicrobial wash to reduce SE on poultry products.

P2-153 Control of *Listeria monocytogenes* in Ham Using Clean-Label Cultured Celery Powder (VegStable® Secure)

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Introduction: Shelf-life extension is a permanent challenge in the ready-to-eat industry, especially when customers today demand understandable ingredients in the ingredient list. This study demonstrates a clean-label antimicrobial powder compared to buffered vinegar powder on *Listeria* control of a ham product.

Purpose: To evaluate the efficacy of a celery-based clean-label antimicrobial on naturally cured ham against aerobic bacteria (APC), Lactic acid bacteria (LAB), and *Listeria monocytogenes*.

Methods: Formed ham formulated with 1.05 – 1.20% cultured celery powder was cooked, sliced, and inoculated with a cocktail of *L. monocytogenes* before vacuum packaging. Each bag contained ten slices of ham stored at 4°C for 120 days. Uninoculated slices were stored under the same conditions to monitor the native flora. At every 15-day time point, two packs of each treatment were tested for the growth of APC, LAB, and *L. monocytogenes.* Ham made with 0.65% buffered vinegar powder was used as positive control; and a treatment with no preservatives added was used as negative control.

Results: Ham treated with cultured celery powder, along with buffered vinegar powder, suppressed the growth of *L. monocytogenes* under two log CFU/g increase for the study duration of 120 days, while the negative control failed after 56 days. The cultured celery powder and positive control treatments preserved APC under 7 log CFU/g for 96 days, which prolonged the ham shelf life by more than eight weeks compared with the negative control. The ham treated with 1.2% cultured celery powder and the positive control both suppressed the LAB outgrowth for the duration of 120 days, while the negative control failed at day 78.

Significance: VegStable® Secure cultured celery powder can match the efficacy of buffered vinegar powder on aerobic bacteria (APC), Lactic acid bacteria (LAB), and *Listeria monocytogenes* in a ham model.

P2-154 The Impact of Essential Oils and Prebiotics on Ileal Microbiota and Blood Metabolites in Late-Laying Hens

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❖ ***Developing Scientist Entrant***

Introduction: Essential oils are increasingly used in the poultry industry as beneficial feed supplements that may be utilized to maintain gut health, promote growth, balance intestinal microflora, and exhibit antimicrobial properties.

Purpose: This study evaluated the effect of feeding essential oils (EO) on late-laying hens and their impact on various blood metabolites and ileal microbiota.

Methods: Sixty commercial caged White Leghorn laying hens were randomly allocated to one of four dietary treatments and fed ad libitum for twelve weeks. These treatments included 0% control (corn–soybean meal-based basal diet), 0.5% (low EO-based basal diet), 1% (high EO-based basal diet) diets, and a 1% prebiotic-based basal diet. Weekly body mass and feed conversion ratio were measured and recorded. This project complies with all IACUC standards. At the end of the twelfth week, a complete blood profile was performed, and ileal contents were collected for microbiota analysis. Blood metabolite data was analyzed using SAS 9.4. For ileal microbiota, the primers (341F/805R) were designed to target the V3 and V4 regions of 16S rDNA from fecal samples and the amplified library is sequenced on a NovaSeq platform.

Results: Metabolite panels were lowered in prebiotic-treated hens. There was a stepwise decrease in Phosphorus and Calcium levels from control to prebiotic diets. The 0.5% low EO-based basal diet treated birds showed a significant increase (p < 0.05) in Lipemic Index compared to other diets. Other metabolites measured showed no significance between treatments (p > 0.05). Amongst all treatments, ileal microbiota exhibited significantly higher rela-

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tive abundance of *Lactobacillus* and *Limosilactobacillus* ($p < 0.05$) compared to other microorganism species.

Significance: Further research is necessary to evaluate essential oils' role as a potential feed additive in the diets of laying hens. The use of EO can competitively exclude the colonization of pathogenic microbes in the gastrointestinal tract, thus enabling safer food production.

P2-155 Assessment of Bacteriophages to Control *Salmonella* Strains of Meat and Poultry Origin

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Undergraduate Student Award Entrant

Introduction: *Salmonella* continues to be a challenge in meat and poultry with lytic bacteriophages (phages) offering a possible specific intervention.

***Purpose*:** Evaluate three experimental phages alone and combined as a cocktail to prevent the growth of *Salmonella* sourced from beef (n=48), pork (n=94), and poultry (n=47 chicken and n=48 turkey).

***Methods*:** *Salmonella* were of 65 serotypes including 17 of concern in meat and poultry that are commonly associated with human illness (Anatum, Berta, Dublin, Enteritidis, Hadar, Heidelberg, Infantis, Montevideo, Muechen, Newport, Reading, Saintpaul, Schwarzengrund, Thompson, Typhimurium, and I,4,[5],12:i:-; n=139). Each strain ($1x10^6$CFU/mL) was exposed to ten-fold serial dilutions (10^{-1}-10^{-6}) of each phage (10^{10} PFU/mL) or the cocktail. The phage exposed *Salmonella* were incubated 4h at 37°C, their growth measured by absorbance (550nm), and compared to no phage control. A multivariate linear model was applied to determine which phage at which dilution performed best for *Salmonella* growth inhibition. The percentage *Salmonella* growth in presence of phage was modeled as a function of serotype, commodity, pathogenicity, and the interaction of the phages and dilution level.

***Results*:** All phage solutions performed significantly differently from one another ($p<0.05$). In all cases, after marginal model predictions calculated for commodity (averaged over serotype and pathogenicity) and serotypes of concern (averaged over commodity and pathogenicity), the cocktail at 10^{-1} ($1x10^9$ PFU/mL) outperformed all other phages and dilutions at limiting *Salmonella* growth. For the commodities, the predicted percent of *Salmonella* strains that grew in the presence of cocktail at 10^{-1} ranged from 7.3% for beef to 12.2% for turkey. For the serotypes of concern, the predicted percent that grew in the presence of cocktail at 10^{-1} ranged from 0% for Berta, Dublin, Enteritidis, Heidelberg, Newport, Schwarzengrund, Typhimurium, and I,4,[5],12:i:- to 36.0% for Thompson.

Significance: Use of this phage cocktail may prove beneficial at reducing particular *Salmonella* strains of concern when applied to fresh meat and poultry.

P2-156 Strategies in Fermented Sausage Safety: A Comprehensive Analysis of Pathogen Inactivation Dynamics

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Developing Scientist Entrant

Introduction: Ready to eat (RTE) fermented sausages have been implicated in foodborne outbreaks.

***Purpose*:** Observing the factors that influence pathogen inactivation is critical for ensuring food safety in fermented sausage production.

Methods: Two types of fermented sausages, summer sausage (semi-dried) and salami (dried), were prepared with recipes from Michigan State University Meat Lab. Sausage batters, inoculated with *Escherichia coli*O157:H7, *Listeria monocytogenes*, *and Salmonella enterica* (3×10^7 CFU/g), were stuffed into various diameter casings and fermented to pH 4.8±0.1. Small sausages (18 and 30mm) were dried and sampled for pathogen concentration, pH, and water activity (a_w) over 30 days, while large sausages (60, 90, and 110mm) were sampled over 60 days in three trials for each sausage type. A non-parametric robust regression assessed the influence of processing steps, aw, pH, and sausage type on the pathogen reduction.

Results: The drying process achieved a greater aggregate reduction of the three pathogens (*E. coli, L. monocytogenes, and S. enterica*) than fermentation, resulting an increase in log reduction of 0.75 ± 0.14 ($p < 0.05$). During fermentation, *S. enterica* had a significantly higher log-reduction of 0.72±0.28 in salami compared to summer sausage ($p <0.05$). During drying, every 10 days of drying resulted in a 0.7±0.07 log greater aggregate pathogen reduction in the small sausages compared to the large sausages ($p <0.05$). However, there were no size-specific differences within each size group. The analysis indicated that both drying days and aw were significantly associated with the aggregated pathogen reduction ($p <0.05$). Specifically, every 10 days of drying was associated with an increase of 0.70±0.04 log-reduction. Similarly, a decrease of 0.1 in a_w corresponded to an increase of 0.94±0.1 in log-reduction ($p <0.05$).

Significance: This study emphasized food safety practices in fermented sausage production, highlighting the critical roles of the operation parameters.

P2-157 Shiga Toxin-Producing Escherichia coli Contamination on the Surfaces of Beef Carcasses in Slaughterhouses in Japan

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Introduction: Beef is a major source of Shiga toxin-producing *Escherichia coli* (STEC) infections. Global beef trade has recently increased in Japan, and confirmation of STEC non-contamination is essential for exporting to foreign countries. However, there are only a few studies on the frequency of STEC in beef carcasses in various slaughterhouses for beef distributed in Japan.

Purpose: The purpose of this study was to assess the potential risks of STEC related to beef carcasses contamination with STEC in slaughterhouses.

Methods: A total of 524 gauze samples were collected from 12 domestic slaughterhouses in Japan. The samples were obtained from distinct cattle carcasses in each slaughterhouse using three sterile gauzes (30 cm × 30 cm) soaked in 50 mL of phosphate-buffered saline (PBS) by swabbing on the surface of beef carcasses between the neck and chest. Modified tryptone soya broth (250 mL) was added to the sample, and the sample solutions were used to measure aerobic plate counts and were enriched, followed by screening of O26, O45, O103, O111, O121, O145, and O157 STEC.

Results: STEC O157:H7 and *stx*-positive *E. coli* were isolated from 0.6% and 4.6% of beef carcass surfaces, respectively. Although the STEC O157:H7 isolation rate was low and *stx*-positive *E. coli* isolates belonged to minor O-serogroups, indicating low pathogenicity in humans, they pose a risk of foodborne illness. Therefore, it is important to re-evaluate hygiene treatments for beef carcasses, particularly in facilities with high aerobic plate counts.

Significance: The isolation rate of STEC O157:H7 was 0.6%, and O26, O45, O103, O111, O121 and O145 were not isolated. These results indicate that the contamination levels of STEC in beef carcasses in Japan is low.

P2-158 Microbiological Quality Assessment of Plant-Based Milk: Multivariate Data Analysis

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Introduction: Bacterial endospores can withstand industrial processing at high temperatures such as UHT, enabling these microorganisms to germinate and proliferate during product storage. Certain *Bacillus* species, for instance, are known culprits of food-borne illnesses, manifesting as diarrheal and emetic poisoning, while others pose concerns primarily due to their role in food spoilage, leading to substantial financial losses in the industrial sector.

Purpose: This study aimed to assess the microbiological quality of plant-based milk derived from diverse food matrices and available in supermarkets in Campinas, SP, Brazil.

Methods: For this purpose, a commercial sterility test for low-acidity foods was conducted according to the methodology recommended by APHA (2015) on 111 samples from 10 different brands, collected across 9 supermarkets. Color changes from purple to yellow were observed in the tubes in at least one of the culture media (DTB or PE-2) and at one of the incubation temperatures (35 °C or 55 °C) in 82 samples, equivalent to approximately 74% of the samples analyzed.

Results: In total, 1221 aerobic spore-forming bactéria were isolated, comprising 764 thermophiles and 457 mesophiles. Utilizing multivariate data analysis of microbial growth, the samples were categorized into 4 classes, with radial visualization further grouping them into 8 classes. Consequently, the industry faces the challenge of developing novel processing strategies for thermoprocessed foods, aiming to minimize and prevent the survival of highly heat-resistant spores.

Significance: Spore-forming bacteria stands out as a significant contributor to spoilage and intoxication, emphasizing the need for targeted interventions in food processing.

P2-159 Emerging Non-Thermal Technologies: Application of Ozone in Microbial Reduction of Beef

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Introduction: Ozone is an allotrope of oxygen with high oxidative power and an excellent sanitizer.

Purpose: Water (OW: vacuum pump/microbubble generator/tank (26 L)/ozone generator) and mist (OM: ultrasonic humidifier/ozone generator) ozonized systems were tested for superficial decontamination of beef.

Methods: Samples of ~100g) were treated by spraying with OW ([O3] =16 mg/L), in a closed system, for an exposure time (ET) of 0.25, 0.5, 1, and 3 min; and to OM for 0.25, 0.48, 1, 3, 6, 12 and 24 min (at 1 and 2 L/min of O_3). Microbiological (lactic acid bacteria-BAL, mesophilic bacteria-MES, enterobacteria-ENT, and *E. coli*-EC) and physicochemical analyses [a_w, pH, color, and lipid oxidation] were carried out.

Results: There was a tendency for populations to be reduced (~1 logarithmic cycle) up to 30 seconds of exposure to OW. The reductions were 2.18, 0.57, 0.52, and 0.61 for the populations of EC, BAL, MES and ENT, without changing the parameters of a_w, pH, L, a, b, c, and TBARS (mg MDA/Kg): 0.993 ± 0.002, 5.55 ± 0.01, 39.48 ± 2.22, 26.70 ± 4.58, 15.19 ± 1.54, 30.02 ± 5.01 and 0.219 ± 0.032, respectively. NO reduced ~0.8-1 log cycles in BAL, MES, and ENT populations at 24min (Q=1L/min). At 2 L/min, there was a reduction of ~2 logs in MES, and 1 log in the other populations, after 24 min. However, at 12 and 24 min, there were changes in the beef color parameters. At 6 min, the values of L, a, b, c, and TBARS (mg MDA/Kg) remained constant (p<0.05): 36.70, 29.61, 16.74, 34.05 and 0 .21, respectively, with an average reduction of 0.3, 0.4 and 0.05 logs of MES, ENT and EC.

Significance: Other studies, involving barrier technologies, must be associated with this, to verify their impact on production, in terms of costs and sustainability.

P2-160 Salmonella Lethality During Pilot-Scale Rotisserie Chicken Roasting

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✦ ***Undergraduate Student Award Entrant***

Introduction: The revised USDA FSIS Cooking Guideline Appendix A identified a scientific gap regarding *Salmonella* lethality on the surface of rotisserie cooked chicken, given the potential for surface drying that may promote *Salmonella* survival.

***Purpose*:** The purpose of this study was to validate *Salmonella* surface lethality during pilot-scale cooking of rotisserie chickens.

***Methods*:** Raw whole chickens (1.36-1.81 kg) were sourced from a local supplier, frozen. The chickens were thawed (4ºC), then surface-inoculated with an 8-strain *Salmonella* cocktail and stored at 4ºC before cooking. Two inoculated and 4 uninoculated carcasses were then cooked in a retail-scale rotisserie oven (18-bird capacity) at 204.4ºC, with no additional water or humidity added to the system (maximum wet bulb of 61.4±1.0ºC), until an internal temperature (at thickest location of the breast) ≥73.9ºC was reached (in triplicate). Skin sections then were excised from the upper/lower anterior and posterior and from the left/right wings and legs – resulting in >95% skin removal for sampling. The excised samples were chilled in peptone water, stomached, serially diluted, plated on differential/non-selective media (modified Tryptic soy agar), incubated (48 h, 37ºC), then enumerated.

***Results*:** The average cook time and yield were 53.8±2.8 min and 77.7±5.2%, respectively. Although some individual samples exceeded 7 log reductions, the mean *Salmonella* log reduction for the upper and lower anterior, wings, legs, upper and lower posterior were 5.73±1.20, 5.84±1.08, 5.70±1.00, 5.83±1.50, 5.55±0.99, and 5.62±1.50, respectively. Sample location did not impact *Salmonella* reductions (α=0.05). None of the sample locations exceeded the seven-log reduction target (α=0.05).

Significance: The results suggest that a rotisserie oven with no additional humidity added, running under-loaded, with less water vapor in the oven than in a fully loaded oven, may result in less than the target 7-log *Salmonella* reductions on the surface of rotisserie-cooked chickens. Further work is needed to evaluate fully loaded oven scenarios.

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P2-161 Salmonella Inactivation in Baked Beef Pasties

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✦ ***Undergraduate Student Award Entrant***

Introduction: Foods with meat encased in dough, such as pasties, have been identified as a "scientific gap" in the USDA FSIS Revised Cooking Guideline Appendix A, indicating a need for additional scientific data.
Purpose: The objective was to quantify the inactivation of *Salmonella* during pilot-scale cooking of pasties.
Methods: Beef cubes (raw and pre-cooked) or dough were inoculated with an 8-strain *Salmonella* cocktail. Pasties then were hand formed using equal portions (~60 cm^3) of raw or pre-cooked beef cubes, potato cubes, and diced onions enveloped in dough. Triplicate batches of four pasties were baked in a small retail oven at 160°C for 30 min (par-bake) or 50 min (full bake). Pasties then were cut into thirds (two edge and a center portion) and quenched in 150 ml chilled peptone water. Samples were stomached, serially diluted, plated on a non-selective/differential medium (modified Tryptic soy agar), incubated (37°C, 48 h), and enumerated.
Results: Initial *Salmonella* levels in inoculated raw beef, pre-cooked beef, and dough were 9.18±0.24, 9.03±0.15, and 7.96±0.47 log CFU/g, respectively. By 30 min cook time, core temperature of beef cubes achieved ≥71°C, but no treatment resulted in ≥6.5 log reduction. By 50 min, *Salmonella* reduction in the raw beef, pre-cooked beef, and dough-inoculated pasties was 8.20±1.29, 7.93±0.6, and 7.32±0.85 log reductions, respectively, exceeding the 6.5 log reduction target for beef products ($p < 0.05$).
Significance: Pasties failed to achieve 6.5 log reductions of Salmonella at the par-cooked state, even though beef cube temperatures reached 71°C; however, those treatments resulted in a visibly undercooked product. By 50 min, pasties resembled a fully cooked, ready-to-eat product and reliably achieved >6.5 log reductions of Salmonella. Starting with pre-cooked filling would reduce the overall risk of pathogens surviving in such products.

P2-162 Enterococcus faecium Can Serve as a Surrogate for the Thermal Lethality of Salmonella in Ground Pork Products

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Introduction: Consumption of contaminated pork and pork products is commonly associated with *Salmonella* infections in humans. Farm-to-table transmission via fecal material during animal production and transportation, and during processing and transformation make *Salmonella* a significant food safety concern in the pork industry.
Purpose: This study aimed to determine the thermal inactivation kinetics of *Enterococcus faecium* and compare it to that of generic *Escherichia coli* and *Salmonella* in raw ground pork.
Methods: Fresh ground pork (ca. 5% fat) was inoculated with *Enterococcus faecium* strain (NRRL B-2354) to a final concentration of 7-8 log CFU/g. Five-gram pouches of the inoculated pork were submerged in a circulating water bath at treatment temperatures of 55, 60, 65, and 68 ℃ for predetermined times. The cells were recovered on KF Streptococcus Agar with 2% of 1% Triphenlytetrazolium Chloride. The Microrisk Lab (version 1.2) fitted a loglinear model to the survival data to obtain the D-value, further analysis was achieved in R (version 4.3.1).
Results: The D-value of *E. faecium* in contaminated pork at 55, 60, 65, and 68 °C was 175.44,13.5, 1.14, and 0.26 min, respectively. The thermal resistance of *E. faecium* was significantly higher ($p<0.05$) than that of *Salmonella* (19.37, 0.66, and 0.16 and 0.19 min respectively) at all heat treatments. Therefore, the strain of *E. faecium* used in this study was a suitable as a conservative surrogate for *Salmonella* at 55, 60, 65, and 68 °C in ground pork.
Significance: These results can be used by industry and regulators to develop, validate, and verify appropriate lethality treatments measures for pork processing operations, thereby decreasing the risk of *Salmonella* infection via consumption of contaminated ground pork products.

P2-163 An Iterative Approach to Identify a Gold-Standard Method for Assessing *Salmonella* Load and Prevalence in Broiler Houses

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❖ ***Developing Scientist Entrant***

Introduction: To improve food safety in broilers, there is a critical need to accurately assess *Salmonella* during pre-harvest.
Purpose: This iterative study evaluated six different environmental sampling methods to measure *Salmonella* prevalence and load in broiler houses across three phases.
Methods: In phase 1 (24 houses/10 farms), bootsocks, electrostatic pads over a paint-roller, feather swabs, cloacal swabs, fecal grabs, and litter grabs were evaluated. Phase 2 (16 houses/7 farms), bootsocks, bootsocks over a paint-roller, and feather swabs were evaluated. Phase 3 (20 houses/10 farms), bootsocks and bootsock-rollers were evaluated in triplicate in each house. In all three phases, samples were collected on both sides of each house independently. *Salmonella* prevalence was determined by both qPCR and culture. *Salmonella* load was inferred by the Ct-values of the primary enrichment. Fisher's Exact test and F-test were performed to establish significant differences among sample types ($p<0.05$).
Results: For phase 1, *Salmonella* prevalence differed among sample types when evaluated by qPCR ($p=0.015$), but not by culture. The best performing sample types were bootsocks (Culture: 42/48, qPCR: 41/48), feather swabs (42/48 and 36/48), and electrostatic pads on rollers (35/48 and 34/48). For phase 2, there were differences by qPCR ($p=0.0004$) but not by culture. Bootsock-rollers (31/32 and 28/32) and traditional bootsocks (31/32 and 28/32) were the best performing. For phase 3, prevalence was higher by qPCR (210/240) than culture (167/240) for both sample types ($p=0.0021$) but there was no difference per sample type or within replicates. The average Ct-value of bootsocks (35.7) was lower than bootsock-rollers (36.4) ($p=0.0002$). A mixed-effect model showed that for *Salmonella* load, 44% of the variance was accounted for by house, and 15% by house side. Variance across replicates was low (4%).
Significance: *Salmonella* recovery is directly influenced by sample type. For environmental sampling of broiler houses, traditional bootsocks and bootsock-rollers provide the best indication of *Salmonella* prevalence and are the most reproducible sample types.

P2-164 Prevalence of Shiga-Toxin Producing *Escherichia coli* in a Meat Processing Facility in the Midwest of the United States: A Longitudinal Study

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Introduction: The lairage area is where animals are held before slaughter. Prevalence in the lairage area is correlated with the number of positives in the final product. Monitoring and controlling prevalence in the lairage area can help to reduce the number of positives in the final product or combos.
Methods: In this study, boot swabs were collected in the lairage area in a meat processing facility in the Midwest for one year (n=15 every month). Boot covers were used in each foot to collect the fecal material from the pens. Swabs were taken walking thought the lairage area in a "Z" pattern and then put into a filtered bag. Subsequently, 100 mL of BPW were added to each boot swab, stomached at 230 RPM for 1 minute, and 30 mL of stomached samples were transferred into a 7 oz sterile bag (Whirl-Pak Bag). Thereafter, 30 mL of BAX MP + 1 mL/L Quant Solution into each sample. Samples were incubated for 24 hours at 42˚C. After incubation was finished, samples were run using BAX Real-Time Assay for *E. coli* O157:H7, Panel 1 and Panel 2.
Results: According to One-Way ANOVA used to analyze the data, there are significant differences between months ($p < 0.05$). This means that the

prevalence of the lairage area varies between months, having the highest prevalence in the warmer months of the year (June – August) and the lowest prevalence in the coldest months of the year (December – February). This variation can be caused by climatic conditions such as temperature, humidity, and precipitation.

Significance: According to the obtained results, targeted interventions can be carried out during the warmer months to reduce the prevalence of pathogens. Monitoring is an important tool to detect and control any potential hazards in a meat processing facility.

P2-165 Antimicrobial Resistant Non-*aureus* Staphylococci in the Pork Production Chain in Korea: High Prevalence of SCCmec uxp and Occurrence of *cfr*-Mediated Linezolid Resistance

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Introduction: Non-*aureus* staphylococci (NAS), particularly antimicrobial-resistant NAS, have a substantial impact on food safety and public health. It has been proposed that antimicrobial-resistant NAS in livestock farms represent a significant reservoir of antimicrobial resistance genes (ARGs).

Purpose: This study was aimed to investigate species profiles of antimicrobial-resistant NAS in the pork production chain in Korea and the genetic determinants for the resistance phenotypes.

Methods: Staphylococci were isolated from pig farms, slaughterhouses, and retail pork samples located in 8 provinces in Korea. NAS were identified based on 16S rRNA and *tuf* gene sequencing. Antimicrobial susceptibility assays were performed using 16 different antimicrobials, including cefoxitin and linezolid. For methicillin-resistant staphylococci (MRS), staphylococcal cassette chromosome *mec* (SCC*mec*) typing was carried out. To analyze the genetic contexts of SCC*mec* and *cfr*, whole genome sequencing analyses were performed on selected MRS and linezolid-resistant strains.

Results: In total, 364 NAS isolates of 17 different species were collected in the pork production chain in Korea. Occurrence of muti-drug resistance phenotypes was identified in 54.9% (n=200) of the NAS isolates with highest resistance rates clindamycin and chloramphenicol (65.9% and 60.4%, respectively). Among the 57 MRS isolates, 50 isolates (87.7%) carried SCC*mec* type V for methicillin resistance. Linezolid resistance was identified in 7.7% (28/364) of NAS isolates, all of which carried *cfr*. Comparative whole genome sequence analyses also revealed that different species of NAS and *S. aureus* shared identical SCC*mec* V and *cfr* elements.

Significance: These findings demonstrate that NAS in pork production chain have a potential role in acquisition and transmission of ARGs, such as SCC*mec* V elements and *cfr* gene.

P2-166 Prevalence and Phenotypic Resistance of *Salmonella enterica* Isolated from Guinea Fowl Wet Markets in One Health Concept

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Introduction: *Salmonella* spp. are important foodborne pathogens responsible for some foodborne outbreaks. Their resistance to antibiotics poses a threat to public health due to therapeutic difficulties and sometimes failures.

Purpose*:* To determine the prevalence and antibiotic resistance of *Salmonella enterica* isolated from guinea fowl wet markets using one health concept.

Methods*:* The procedure of the USA-FDA BAM was used to isolate *Salmonella enterica*. Twenty-five samples each of faeces, meat, processing knife, processing floor, processor's hands, processing table, water used for washing meat and water from main source were sampled from guinea fowl markets in Tamale metropolis, Ghana. The disc diffusion method was used for the antibiotic resistance test.

Results*:* The prevalence of *Salmonella enterica* was 6.5% (13/200). *Salmonella enterica* were isolated from water used for washing meat (16%), processing knife (12%), processing floor (8%), meat (8%) and faeces (8%), but not processor's hands, processing table and water from main source. The *Salmonella enterica* exhibited high resistance to azithromycin (73.4%). Intermediate resistance was relatively high for tetracycline (53.3%), gentamicin (40%) and chloramphenicol (40%). Multidrug resistance (resistance to ≥3 different classes of antibiotics) was found in 46.7% of the isolates. The percentage resistance to two, one and zero antibiotics were 13.3%, 33.3% and 6.7%, respectively. One *Salmonella enterica* each isolated from water used for washing meat (ceftriaxone-azithromycin-gentamicin-tetracycline) and processing floor (azithromycin-chloramphenicol-tetracycline-amoxycillin/clavulanic acid) was resistant to as many as four different antibiotics. All the *Salmonella enterica* isolates were susceptibility (100.0%) to ciprofloxacin (100%) and suphamethoxazole/trimethoprim (100%). Susceptibility was also high for amoxicillin (80%) and amoxycillin/clavulanic acid.

Significance: Some of the guinea fowls sampled from the wet markets were contaminated by *Salmonella enterica* which exhibited different antibiotic resistant patterns. The consumption of improperly cooked guinea fowl meats will pose a threat to public health. Proper cooking of guinea fowl meats and measures require to prevent cross contamination are warranted to prevent foodborne infections.

P2-167 How Would You Like Your Tacos? Plain, with Salsa, with Vegetables, and/or *Salmonella?*

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✦ ***Undergraduate Student Award Entrant***

Introduction: Tacos are one of the most consumed culinary delights in Mexico and around the World. Nevertheless, this dish has recently been associated with an outbreak of salmonellosis.

Purpose: This study aims to assess hygiene practices and prevalence of *Salmonella enterica* in pastor tacos sold by street vendors in Querétaro, Mexico.

Methods: A total of 85 street taco establishments were visited. An in-depth evaluation of vendor management and hygiene practices was undertaken using a questionnaire comprising 28 items ranging from 2 (worst) to 5 (best). Additionally, the presence of *S. enterica* was investigated in pastor tacos, in both cooked and raw salsas and vegetables, using the methodology proposed by Bacteriological Analytical Manual of the FDA.

Results: Among the management and hygiene practices observed in the 85 street establishments, it was noted that the implementation of certain practices such as the use of gloves (2.0 ± 0.0), water supply (2.94 ± 0.95), monitoring temperature (3.03 ± 0.86), and separation of cookware (3.57 ± 0.65) were less frequently observed. A total of 85 pastor tacos, 34 green salsas (raw and cooked), 53 red salsas (cooked), and 81 vegetables (coriander and onion) were analyzed. The prevalence of *S. enterica* was found to be highest in red salsa (7.54%), followed by green salsa (2.94%), vegetables (2.46%), and pastor tacos (1.18%). Notably, the only green salsa contaminated was from one of the street establishments where also red salsa was contaminated.

Significance: The absence or inadequacy of certain hygiene practices in street taco establishments, such as lack of separation of kitchen utensils, insufficient water supply, and lack of temperature monitoring, could be linked to *S. enterica* contamination. The salsas were the items most associated with the presence of this pathogen, potentially contaminating the final product as they are commonly consumed together.

P2-168 Radio Frequency (RF) Plus Heat for In-Shell Egg Pasteurization

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Introduction: Less than 3% of the eggs in the United States are sold pasteurized because of the long thermal process (> 60 min) and adverse effects on quality.

Purpose: This research studied the inactivation of *Salmonella* Typhimurium inoculated in eggs using a new generation of an RF pasteurizer, evaluating the overall quality of the eggs after processing.

Methods: Large white eggs (58.3 g) were inoculated inside with *S.* Typhimurium (10^5 CFU/egg) and processed with a new RF pasteurizer that operates at 40 MHz, 35 W for 4.5 min (T_{water} = 38.5°C, 14 l/min) followed by hot water spray at 56.7°C (20 min). The final step was ice water or forced air cooling (30 min). Microbial counts were evaluated after processing, and the presence of sub-lethally injured cells (TSAYE + 3% NaCl) and cell recovery after seven days (7°C) were assessed. The overall quality of eggs was studied in terms of yolk color, Haugh unit, yolk index, shell breakage strength, yolk and albumen pH, and albumen degradation. Experiments were conducted at least twice with triplicate samples in different weeks. ANOVA one–way was used to find significant differences (α = 0.05).

Results: The RF plus heat system was able to pasteurize the eggs after 24.5 min reaching a 5-log reduction regardless of the cooling method. No sub-lethally injured cells were detected in any of the samples. Eggs were analyzed after 7 days for cell recovery, and no viable cells were detected (Limit of detection <1 log CFU/g). Regarding the quality, most attributes were like the control eggs. However, albumen degradation showed a significant difference (p<0.05) between control and RF–treated eggs.

Significance: The new generation of the RF pasteurizer represents a potential option to ensure the food safety of in-shell eggs for special markets, *preserving* their quality.

P2-169 Growth Kinetics of *Listeria monocytogenes* on Chopped Citric Acid-Treated Hard-Boiled Eggs

Megan Fay[1], Aishwarya Marathe[2], Bashayer Khouja[1], Joelle K. Salazar[1] and Diana Stewart[1]

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Introduction: Hard boiled eggs (HBEs) are commercially treated with citric acid and available to consumers at retail. HBEs can be chopped and held prior to consumption or incorporation into multi-commodity foods. While it is known that *L. monocytogenes* can survive on citric acid-treated HBEs during long term storage, no information is available on the survivability of the pathogen on HBEs once chopped.

Purpose: To examine the growth kinetics of *L. monocytogenes* on citric acid-treated or water-treated chopped HBEs during 5, 10, or 25ºC storage.

Methods: Eggs were boiled, peeled, and treated by submersion with either water or a 2% citric acid solution for 24 h. HBEs were then each inoculated with *L. monocytogenes* at 4 log CFU. HBEs were chopped and stored in deli containers at 5, 10 or 25ºC for up to 14 d with enumeration during storage. Three independent trials were conducted. *L. monocytogenes* growth rates were determined using DMFit. Statistical differences between growth rates were determined via ANCOVA; p≤0.05 was considered significant.

Results: All *L. monocytogenes* growth rates were significantly higher on the water-treated chopped HBEs than on the citric acid-treated at all storage temperatures. Growth rates at 5°C were 0.08±0.02 and 0.02±0.02 log CFU/g/d on the water and citric acid-treated samples, respectively. At 10°C, growth rates were 1.03±0.09 and 0.54±0.07 log CFU/g/d, respectively, resulting in 1 log CFU/g population increases in 0.97 and 1.85 d. Significantly higher growth rates were observed at 25°C; growth rates were and 4.38±0.23 and 1.40±0.18 log CFU/g/d, respectively, resulting in 1 log CFU/g increases in only 0.23 and 0.71 d.

Significance: The results of this study aid in understanding the growth of *L. monocytogenes* on chopped HBEs and can inform guidance on proper time and temperature combinations for safety.

P2-170 Survival of *Listeria monocytogenes* in Deli Salads Containing Hard-Boiled Eggs Treated with Citric Acid

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❖ ***Developing Scientist Entrant***

Introduction: Recent listeriosis outbreaks and recalls have been associated with commercially available hard-boiled eggs (HBEs). These HBEs are commonly treated with citric acid prior to retail availability and can be left whole or chopped for incorporation into deli salads. This study evaluated the survival of *Listeria monocytogenes* in deli salads made with citric acid-treated HBEs.

Purpose: To examine the survival of *L. monocytogenes* during storage of four different deli salads prepared with citric acid-treated or water-treated HBEs.

Methods: HBEs were treated with 2% citric acid or water by submersion for 24 h at 5°C. HBEs were dried 10 min, inoculated with *L. monocytogenes* at 1 log CFU/HBE, and allowed to dry for 10 min. HBEs were chopped and incorporated into potato, macaroni, chicken, or tuna salad at 1:6 (HBE to other ingredients). Salads were stored at 5, 10, or 15°C for 28 d. *L. monocytogenes* was enriched and/or enumerated throughout storage. Three independent trials were conducted with triplicate samples at each timepoint (n=9).

Results: The initial population of *L. monocytogenes* in the deli salads was 2.01±0.47 log CFU/25g. After 28 d at 5 or 10°C, populations in all salads were <1.70 log CFU/25g regardless of egg treatment, however the pathogen was always detected via enrichment (9/9). After 28 d at 15°C, the population significantly increased in all salads, except for tuna salad when HBEs were citric acid-treated. Populations increased by 1.74, 1.31, 1.03, and 0.63 log CFU/25g in potato, macaroni, chicken, and tuna salad when HBEs were water-treated and by 1.58, 1.36, and 0.53 log CFU/25g in potato, macaroni, chicken salad when HBEs were citric acid-treated.

Significance: This study suggests that HBE treatment, temperature, and deli salad ingredients all play a role in the survival and/or growth of *L. monocytogenes* and all of these variables should be considered for the safety of these products.

P2-171 Risk Factors Associated with *Salmonella enterica* and *Campylobacter* spp. Prevalence among Backyard Poultry in Vermont

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❖ ***Developing Scientist Entrant***

Introduction: Backyard poultry ownership and associated zoonotic illnesses have increased, but the prevalence of and risk factors for pathogen carriage in backyard poultry are poorly understood.

Purpose: To determine the prevalence and risk factors for *Campylobacter spp.* and *Salmonella enterica* in backyard flocks in Vermont in 2022-2023.

Methods: Bedding samples were collected from backyard flocks and farm characteristics recorded. *S. enterica:* 25-gram samples were stomached and enriched in 100mL buffered peptone water (BPW; 4 hours, 37°C). BPW was inoculated into Tetrathionate broth (24 hrs, 37°C), then streaked onto XLT4 or *Salmonella* (Nontyphoidal) Chromogenic Plating Medium agar (48 hours, 37°C or 35°C, respectively), followed by PCR for *hilA*. *Campylobacter spp:* 5-gram samples were immediately inoculated into Cary-Blair transport medium, then inoculated in 20mL Bolton Broth (48 hours, 42°C), followed by detection with the Hygiena Bax *Campylobacter* real-time PCR kit (Bax Q7). Significant differences were assessed using Fisher's Exact tests with the Benjamini-Hochberg correction in R Studio (v.2022.12.0; p<0.05).

Results: We sampled 68 farms. Overall, 13.2% (9/68) and 16.9% (11/68) were positive for *S. enterica* and *Campylobacter spp.,* respectively. *S. enterica* prevalence decreased from 20.6% to 10.8% in 2023 vs 2022, while *Campylobacter spp. p*revalence increased from 12.9% to 17.5%. Two farms were sampled both years. In 2022, neither had *S. enterica* or *Campylobacter spp.* (second farm not tested). In 2023 both farms were positive for both pathogens. A larger flock size was non-significantly correlated with both *S. enterica* (average: 32.6 birds vs 23.4) and *Campylobacter spp.* (average: 52.2 birds vs 23.4) presence. Multiple poultry species on site increased risk of *Campylobacter spp.* Infection (7/11 farms; p=0.002). Three multi-species flocks were co-infected with both *S. enterica* and *Campylobacter* spp.

Significance: The prevalence of both S. enterica and Campylobacter spp., varied by year, and key risk factors were flock size and multi-species flocks.

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P2-172 Occurrence and Antimicrobial-Resistance Patterns of *Salmonella* spp. Isolated from Animal-Origin Food Products Sold at Retail in Chile

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Introduction: *Salmonella* spp. is one of the leading causes of foodborne illnesses, with poultry meat serving as a primary source of transmission. Furthermore, there is a growing incidence of multidrug-resistant (MDR) strains of *S.* Infantis harboring a megaplasmid (pESI), posing a significant global public health concern.

***Purpose*:** To investigate the prevalence and phenotypic antimicrobial resistance profiles of *Salmonella* spp. isolated from retail-sold chicken, turkey, and pork meats, as well as eggs in Chile.

***Methods*:** Over a period of 4 months, 100 retails in the capital city of Chile were surveyed. In each visit, samples from chicken meat (24), turkey meat (5), and pork meat (6), as well as eggs (6) were collected. For the isolation of *Salmonella* spp., traditional methods were used, and presumptive *Salmonell*a colonies were confirmed through *invA*-PCR. Serogroup determination was conducted with a multiplex PCR. The strains were assessed for antimicrobial susceptibility using the disk diffusion method. A PCR targeting the pESI plasmid was conducted on resistant strains.

***Results*:** The percentage of positive samples for *Salmonella* in chicken meat was 49% (47/96), in turkey meats was 10% (2/20), while in pork and eggs, no positive samples for *Salmonella* were detected. A total of 113 *Salmonella* isolates were obtained from chicken meats and 3 in turkey meats. All isolates from chicken meats were of the serogroups C1 and positive for the pESI plasmid. Among these isolates, 12% (12 out of 113) exhibited resistance to ampicillin, 6% (7 out of 113) to cefepime, and 39% (45 out of 113) to cefotaxime.

Significance: This study demonstrates the importance of regular retail food surveillance for identifying and characterizing antimicrobial resistance in foodborne pathogens. The elevated prevalence of third-generation cephalosporin-resistant *Salmonella* contamination in chicken meat not only poses a health risk to consumers but also complicates the treatment of salmonellosis cases.

P2-173 *Salmonella* Quantification (SalQuant®) at 1 CFU/g with Hygiena's BAX® System for Raw Chicken Breast

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Introduction: Component 3 of the proposed USDA-FSIS regulatory framework to reduce the risk of salmonellosis attributed to poultry products will enforce a final product standard based on quantification. The set limit in final product is expected to be 1 colony forming unit (CFU) of *Salmonella* per gram.

Purpose: To meet the proposed USDA FSIS policy, a real-time PCR assay was evaluated for the full quantification of *Salmonella* from 1 CFU/g–10,000 CFU/g in raw chicken breast destined for not-ready-to-eat (NRTE) breaded and stuffed chicken products.

Methods: Raw chicken breast (pre-screened negative) was divided into 16 x 325 g samples and inoculated with a cold-stressed culture of *Salmonella* across 5 log levels. Samples were homogenized with 1,625 mL of BPW as described in the USDA FSIS MLG 4.14. A secondary set of samples was created by transferring 30 mL of the BPW homogenate and combining with equal parts of pre-warmed (42 °C) MP media. Both sample sets were incubated and tested by real-time PCR. Data was analyzed using regression analysis to find the best-fit line. Final estimations were compared to MPN results.

Results: For 325 g samples, the 8-hour enrichment produced the best fit with an R^2 of 0.91 and Log RMSE of 0.46. For 30 mL samples, the 6-hour enrichment produced the best fit with an R^2 of 0.90 and log RMSE of 0.47. There were no statistical differences between MPN values and SalQuant® estimations for all levels except 30 mL samples at 3.00 log CFU/g. SalQuant was superior compared to MPN for this level.

Significance: The BAX® System SalQuant is a sensitive, specific and accurate real-time PCR method that will meet regulatory requirements of quantifying *Salmonella* at levels as low as 1 CFU/g.

P2-174 Genome-Based Machine Learning for Predicting Antimicrobial Resistance in *Salmonella enterica* Isolated from Chicken

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial resistance (AMR) continues to pose a threat to public health. Together, whole genome sequence and phenotypic data provide definitive information on AMR. Machine learning can be used to analyze this data to predict resistance rates and patterns and eventually improve AMR surveillance systems. However, this approach has not been explored by many studies.

Purpose: The objective of this study was to develop a genome-based machine learning model to predict AMR in *Salmonella* isolated from chicken meat.

Methods: Genomic information on 205 *Salmonella* isolates from chicken was combined with the AMR phenotype data of these isolates to amoxicillin-clavulanic acid, ampicillin, ceftiofur, ceftriaxone, sulfisoxazole, streptomycin, tetracycline, and cefoxitin. Four machine learning algorithms were trained on this data to build predictive models. The best-performing model for each antimicrobial agent was used to predict the AMR phenotypes of a new set of 200 *Salmonella* isolates from chicken, and the predictions were compared to AMR phenotype predictions from ResFinder (an AMR genes resource).

Results: The machine learning models showed high sensitivity (>83%), specificity (>83%), and balanced accuracy (>87%) across all the tested antimicrobials. The *sul2* (sulfonamide resistance protein), *blaCMY-2* (beta-lactamase inactivation enzyme), and *tet (C)* (antibiotic efflux pump) genes were identified as some of the important genes used by the models in their classification. The models predicted resistance rates ranging from 1% (amoxicillin-clavulanic acid; ceftriaxone) to 65.5% (streptomycin). When the predictions of the machine learning models were compared to predictions from ResFinder, the predictions from this study were accurate (> 95%).

Significance: These findings highlight the applicability and reliability of machine learning approaches in understanding AMR and predicting resistance patterns, offering a valuable tool in combatting this global health threat. The integration of such predictive models into surveillance systems could significantly enhance our ability to address and mitigate the growing concerns of AMR.

P2-175 Multilocus Variable-Number Tandem-Repeat Analysis Genotype Diversity, Pathogen-Related Genes and Antimicrobial Susceptibility of Enterohemorrhagic *Escherichia coli* Isolates in the Same Food Sample

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Introduction: Various strains of enterohemorrhagic *Escherichia coli* (EHEC) contaminate food, leading to the cause of foodborne infection; however, genotype diversity of EHEC strains isolated from the same food sample, and virulence factors and antimicrobial susceptibility in the strains have been unclear.

Purpose: The study aims to investigate diversity of multilocus variable-number tandem-repeat analysis (MLVA) type among EHEC isolates from the same food sample and analyze virulence factors and antimicrobial susceptibility of these isolates.

Methods: A total of 214 EHEC strains originating from different food samples food samples A and B associated with a foodborne outbreak, and beef samples C, D, E, F, and G were examined. DNA extracted from each strain was used for multiplex PCR for MLVA analysis. PCR assays for *stx* subtypes and

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major virulence factors, and antimicrobial susceptibility test were performed with representative strains from each beef sample.

Results: In samples A and G, EHEC strains from each sample showed same MLVA type and single locus variants. In samples B, C, D, E and F, strains from each sample showed the same MLVA type. Strains from samples C, D, E, F, and G possessed *eae, espB, espD, espP, tir, ehxA, katP, and stcE.* Additionally, strains from sample C possessed *stx2a* and *stx2c*, strains from sample D and E possessed *stx2a* and *iha*, and strains from sample F and G possessed *stx2c and iha*. Strains from sample C were resistant to four antimicrobial agents, including streptomycin and strains from other samples were not resistant to all tested antimicrobial agents.

Significance: This study suggests that EHEC strains showed almost same MLVA type in the same food sample; few EHEC strains isolated from beef samples have antimicrobial resistance; the contamination of strains possessing virulence factors may have the potential to cause foodborne infection.

P2-176 Comparative Effectiveness of Cloth Sampling to Rinse Sampling on Microbial Recovery and *Salmonella* Detection in Poultry Meats

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Introduction: The rinsate method, as proposed by the United States Department of Agriculture (USDA), is the prevailing sampling technique for poultry, but the meat products tested by the rinsate method become inedible after sample collection, which leads to financial loss and food waste. In response, a cloth sampling tool, a MicroTally® Mitt, has been developed to obtain samples without compromising the edibility of meat products.

Purpose: The project aimed to compare the efficacy of mitts and rinsate method on chicken wings and skinless chicken thighs regarding *Salmonella* prevalence, aerobic bacterial counts (APC), and coliform bacterial counts (CBC).

Methods: Sixty chicken wings and sixty chicken thighs were sampled by two methods as separate sample sets. The least squares mean and standard error of the mean for bacterial populations were calculated. The differences between two sampling methods on bacterial populations were evaluated using Analysis of Variance, followed by Tukey's test. The effect of sampling methods on *Salmonella* prevalence was assessed using Fisher's exact test. Statistical analyses were conducted using R software with a significance level of 0.05.

Results: The results revealed that mitts deliver consistent ($p > 0.05$) results in detecting *Salmonella* and CBC compared to the USDA rinsate method. In addition, slight differences were observed in APC ($p < 0.05$) between the two methods, with variations dependent on the specific chicken part examined. Mitts (1.97±0.11 log CFU/g) captured higher APC than rinsate (1.52±0.12 Log CFU/g) for chicken wings, but rinsate (5.9±0.1 Log CFU/g) yielded higher APC than mitts (5.55±0.12 log CFU/g) for thighs. However, the magnitude of these differences did not hold biological significance.

Significance: The findings indicate that mitts may offer a cost-effective alternative to the USDA rinsate method for periodic sampling of chicken parts, enhancing meat product safety control and foodborne bacteria detection.

P2-177 Development of a Multiplex Real-Time PCR Assay for the Detection of Highly Pathogenic *Salmonella* enterica (HPS) in Beef and Poultry

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Introduction: The USDA and industry work to find a balance to continue to offer wholesome, nutritious meat products while reducing risk of illness from *Salmonella* for humans. There is a need in the poultry and beef industries for an easy-to-use method to identify the risk of *Salmonella* in food samples based on virulence or pathogenicity, instead of simply presence or absence.

Purpose: This work involved the development of a real-time PCR assay to detect *Salmonella* in relevant food samples based on genes associated with pathogenicity.

Method: This multiplex PCR assay is designed with three HPS targets (HPS-B, HPS-A, HPS-X) associated with virulence, and combinate with a *Salmonella* spp. target and an internal control. The assay was tested on 60 samples, both enrichments and isolates, from each of the 4 major commodities: chicken, turkey, pork, and beef, and compared to data gathered by USDA-FSIS.

Results: Specificity studies yield 100% inclusivity on 202 *Salmonella* serovars and 100% exclusivity on 53 closely related species of *Enterobacteriaceae*. Of the 129 isolates evaluated from the chicken, turkey, pork and beef samples, the multiplex real-time PCR assay result agreed with the USDA data for the HPS-B, HPS-A, HPS-X, and *Salmonella* spp. targets with an accuracy of 99.2%, 98.4%, 99.2%, and 100%, respectively.

Significance: The results support the assumption that highly pathogenic *Salmonella* can be quickly detected in meat and poultry with this next-generation method for *Salmonella* detection. This approach to *Salmonella* testing can enable the industry to make more risk-based decisions about their product to reduce the number of illnesses caused by *Salmonella* while continuing to provide consumers with nutritious and affordable meat.

P2-178 Validation of Hygiena's BAX® System Real-Time PCR Assays for Salmonella, STEC Suite and E. coli O157:H7 Exact for the Detection of Salmonella and Shiga-Toxin Producing Escherichia coli (STEC) from Beef Trim Sampling Cloths

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Introduction: There is a need in the beef industry for a dependable, easy-to-use pathogen detection method that can detect STEC and *Salmonella* from sampling cloths used to swab beef trim. Hygiena's BAX® System and Fremonta's MicroTally® Manual Sampling Device provide the industry with a method that rapidly provides reliable results.

Purpose: This AOAC matrix extension study assessed real-time PCR assays for detection of *E. coli* O157:H7, STEC and *Salmonella* in sampling cloths swabbed from 375 g beef trim portions.

Method: Forty sampling cloths used to swab 375 g beef trim test portions were fractionally inoculated with either *E. coli* O157:H7 (N=20), STEC (*E. coli* O26:H11) (N=20) or *Salmonella* Typhimurium (N=20) and held at 4 °C for 48 hours. Sampling clothes were then either enriched with 200 mL of pre-warmed (42 °C) MP media or mTSB+caa. All samples were incubated at 42 °C for 8-24 hours and tested using real-time PCR. Results were confirmed using the appropriate USDA MLG confirmation method.

Results: In samples enriched with MP media, 10 of 10 (*E. coli* O157:H7) were detected at 10 and 24 hours; 9 of 9 (STEC) at 8, 10 and 24 hours; and 10 of 10 (*Salmonella*) at 10 and 24 hours. Using mTSB+caa, 8 of 8 (*E. coli* O157:H7) at 8 and 24 hours; 13 of 13 (STEC) at 8 and 24 hours; and 10 of 10 (*Salmonella*) positives were detected at 8, 10 and 24 hours. All positives were confirmed by the appropriate reference confirmation method.

Significance: The real-time PCR assays evaluated allow users to obtain presumptive positive results for *Salmonella,* STEC, and *E. coli* O157:H7 from one 8-10 hour enrichment after processing and PCR analysis.

P2-179 Detection of *Salmonella* and *Listeria* from Large Test Portions of Whole Powdered Egg Using Hygiena's BAX® System Real-Time PCR Assays

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Introduction: Eggs and food products made with eggs can become contaminated at any stage, making these products high risk. Pasteurization is widely used to decontaminate eggs and reduce associated hazards, although some heat-resistant pathogens such as *Salmonella* and *Listeria monocytogenes* may survive.

Purpose: The performance of a commercial real-time PCR assay for *Salmonella* and *Listeria species* was assessed for whole powdered eggs in separate matrix validations and compared against the USDA FSIS reference methods.

Methods: Matrix validations for *Salmonella* and *Listeria* were prepared separately by inoculating bulk portions of whole powdered eggs with an enumerated lyophilized culture at a low fractional level and a high level. Following a 2-week stabilization at room temperature, bulk samples were enumerated to confirm desired target levels. For *Salmonella,* test method samples (375 g) and reference method samples (100 g) were enriched in BPW. For *Listeria,* test method samples (125 g) were enriched in 24 LEB Complete and reference method samples (25 g) were enriched in UVM followed by MOPS-BLEB. Samples were assayed using real-time PCR and confirmed by culture using the appropriate USDA FSIS method.

Results: Real-time PCR returned results for *Salmonella* and *Listeria* with 100% agreement to culture. The probability of detection (POD) determined there were no differences between the test method and reference method for either organism.

Significance: These results demonstrate that the BAX® System Real-Time PCR method is sensitive, specific and accurate for the detection of *Salmonella* in 375 g samples and *Listeria* in 125 g samples of whole powdered eggs.

P2-180 Use of Qualitative and Quantitative Microbial Data to Determine if Turkey Pre-Chill and Post-Chill Sampling are Predictive of *Salmonella enterica* Contamination in Ground Turkey

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❖ ***Developing Scientist Entrant***

Introduction: Understanding the relationship between indicator organism levels (i.e., process controls) and *Salmonella* contamination in ground turkey can provide a proxy test to assess early-processing food safety interventions and predict changes in ground turkey *Salmonella* test results.

Purpose: To evaluate the effectiveness of testing microbial indicators from pre-chill (hot re-hang) and post-chill turkey carcass samples in assessing interventions and predicting *Salmonella* test results in ground turkey.

Methods: A total of 50 hot rehang carcass swabs, 50 post-chill carcass swabs, and 20 ground turkey samples were collected from a commercial turkey processing facility. For each sample, enrichments and serial dilutions were created. From serial dilutions, 1 mL aliquots were plated onto 3M petrifilms to enumerate Enterobacteriaceae (EB), *E. coli* (ECC), and Aerobic Counts (AC). The Hygiena SalQuant™ Real-Time PCR assay was utilized for *Salmonella* detection and quantification.

Results: Based on descriptive statistics, the average log CFU/ml from hot rehang samples was 3.48, 2.81, and 2.25 for APC, EB, and EC, respectively. Alternatively, the average log CFU/ml for post-chill samples was 0.25, 0.14, and below the quantifiable limit for APC, EB, and ECC, respectively. This data demonstrates a discernible reduction in microbial indicators between the two processing locations. *Salmonella* was only detected from one hot rehang (0.44 log CFU/ml) and one post-chill sample (innumerable), corresponding to the same flock which yielded a negative *Salmonella* test result in grind. Moreover, *Salmonella* was only detected in three ground turkey samples (-0.38 log CFU/ml).

Significance: Preliminary results indicate that, on average, a 2-log reduction was observed between hot rehang and post-chill samples. *Salmonella* detection was relatively low at both locations, with 2% (2/100); within ground turkey samples, the prevalence was 15% (3/20). Although additional data will be collected to fully evaluate correlations between hot rehang and post-chill indicator levels and final ground turkey *Salmonella* test result, these data do not appear to indicate a relationship.

P2-181 Utilizing Pre-Harvest Detection and Enumeration of *Salmonella* for Ground Turkey Production

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❖ ***Developing Scientist Entrant***

Introduction: Ground turkey is a critical reservoir for *Salmonella*. Currently, there is no established sampling methodology for *Salmonella* testing in pre-harvest and early-harvest for ground turkey production.

Purpose*:* To characterize *Salmonella* contamination from pre-harvest, early-harvest, and ground turkey production utilizing detection and enumeration methodologies.

Methods*:* A total of 4,025 pre-harvest and early-harvest samples were collected from 66 turkey flocks throughout 12 sampling visits. Specifically, samples from turkey barns included fan dust swabs (3), feather samples (3), and bootie pairs (2). Samples from turkey processing included ceca (10), livers (10), lungs (10), feet swabs (4), vent feather swabs (2), trailer drag swabs (15), and ground turkey (2). Samples were analyzed for *Salmonella* detection and quantification utilizing Hygiena's BAX® System Real-Time PCR assay and BAX® System SalQuant®, respectively. A total of 772 positive samples were evaluated for quantification.

Results*:* Descriptive analyses showed high variability between sample types in *Salmonella* detection and quantification. Vent swabs had the highest prevalence of *Salmonella* (52.89%; 64/121), while ceca samples had the lowest (2.9%; 18/620)—excluding feather samples, which never yielded a positive. A substantial proportion of samples (295/772) were positive for *Salmonella* but with levels below the limit of quantification (1 CFU/unit). On average, quantifiable *Salmonella* values per sample type were approximately 1 log CFU/unit. For ground turkey, 28% (34/123) of samples tested positive, with a mean of 0.41 log CFU/unit and a Standard Deviation of 0.92 log CFU/unit.

Significance: Results depict substantial variability in *Salmonella* detection and quantification across sample types in pre-harvest, early harvest, and ground turkey production. A considerable number of positive samples were either below the limit of quantification or below 1 log CFU/unit, suggesting that despite a rather pervasive presence, levels of *Salmonella* seemed to be predominantly low. Further research is necessary to evaluate correlations between ground turkey and the other samples.

P2-182 Quantification of *Salmonella* spp. and *Campylobacter* in Poultry Carcasses Collected at Different Processing Stages with Reduced Chemical Levels in a Commercial Broiler Facility to Validate the Performance of a Physical Intervention

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Introduction: When equipment modifications targeting fecal contamination removal are considered in a poultry processing facility, bio-mapping based on pathogen quantification may identify the contribution of the modified process to reduce the risk of foodborne pathogens.

Purpose: Develop poultry bio-mapping data based on quantification and detection of *Salmonella* spp. and *Campylobacter* under reduced chemical intervention levels for the side-to-side comparison of two different processing lines, line 2 modified with a fecal-removing device.

Methods: Whole chicken carcass rinses (n=144) were collected from post-pick (PP), rehanger (R), post-ventor (PV), before IOBW (IOBW), pre-chill (PE), and post-chill (PC). BAX®-System-SaltQuant™ and BAX®-System-CampyQuant™ were used to quantify and detect *Salmonella* spp. and *Campylobacter*. BAX®-System-*Salmonella* and BAX®-System-*Campylobacter* prevalence analysis was performed on negative enrichments after quantification. Results were reported as log CFU/sample followed by Wilcoxon Rank sum test (p<0.05).

Results: For *Salmonella* spp., higher loads were found at PP (6.18 and 5.24 log CFU/sample, for lines 1 and 2). Lower loads were shown after PC (0.10 and 0.60 log CFU/sample, for lines 1 and 2). *Salmonella* spp. prevalence was 100% in all the locations except at PC, where lines 1 and 2 had a prevalence of 33.33% and 16.67%. For *Campylobacter*, a significant difference (p>0.05) between lines 1 and 2 at PE (3.96 and 2.48 log CFU/sample) and PC (0.89 and 0.05 log CFU/sample) was observed. *Campylobacter* prevalence in line 1 had a lower prevalence than line 2 at R (66.67% vs 90.91) and IOBW (83.33% vs 91.67%). Line 2 presented lower prevalence than line 1 at PP (75% vs 50%) and PC (66.67% vs 16.67%).

Significance: Using quantitative bio-mapping data on pathogens at different stages of processing can help determine the efficacy of processing modifications including chemical and physical interventions. Using reduced chemical interventions when feasible helps identify the effect of modifications, reducing the masking effect of interventions.

P2-183 Rapid Quantification of *Salmonella* in Chicken Carcass Rinses Using Loop Mediated DNA Amplification (LAMP) Assay

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Introduction: Over 23% of *Salmonella* illnesses are associated with consumption of poultry products. In addition to prevalence, the quantity of *Salmonella* in food can impact the likelihood of causing illness. FSIS is considering regulatory framework for *Salmonella* quantification in raw poultry rinse samples and plans to extend to other sample types. Using Molecular Detection Assay 2 (MDA2) *Salmonella* kit, a customized testing scheme/algorithm was developed to rapidly quantify *Salmonella* in chicken carcass rinses. The LAMP-based isothermal assay amplifies *Salmonella* target-DNA with high specificity and sensitivity.

Purpose: Evaluate a rapid new *Salmonella* quantification method for whole chicken carcass rinses using MDA2 *Salmonella* kit compared to traditional most probable number (MPN) estimation method.

Methods: The chicken carcasses were spiked with four strains of *Salmonella* cocktail and refrigerated for 2-3 days, then rinsed with 400 mL nBPW. For the rapid quantification method, 30 mL of the rinsate was enriched with 30 mL of proprietary medium at 42°C for 6h. After a simple preparation step, it was analyzed with MDA2 *Salmonella* method. The concentration of *Salmonella* was calculated using the new algorithm for quantification. Additionally, the chicken rinsate was tested with the traditional MPN method to determine the amount of *Salmonella* in the sample. Two samples t-test was conducted for statistical analysis to compare the difference in means between the two methods.

Results: The rapid method and algorithm successfully quantified *Salmonella* in chicken carcasses, following 6h enrichment. The mean difference between the MDA2 *Salmonella* rapid quantification and reference MPN method was 0.113 log, which was within 0.5 log, indicating no significant difference when compared to the reference method.

Significance: The MDA2 *Salmonella* test workflow enables a faster and less laborious method to determine the concentration of *Salmonella* in bird rinses, allowing poultry producers to institute appropriate early interventions to minimize the risk of illness.

P2-184 *L. monocytogenes* Colony Confirmation Using GENE-UP® LIS and LMO in Liquid Whole Eggs and Several Ready-to-Eat (RTE) Foods

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Introduction: *Listeria monocytogenes* infections from consuming ready-to-eat (RTE) foods and egg products remains a significant cause of foodborne illness. A critical step toward *L. monocytogenes* management in foods is using rapid and reliable molecular tests alongside traditional culture-based methods as that of the United States Department of Agriculture Food Safety and Inspection Service Method Laboratory Guidebook (USDA-FSIS MLG).

Purpose*:* In a series of verification studies, isolated *L. monocytogenes* colonies were confirmed from selective chromogenic agars using Real-Time Polymerase Chain Reaction (RT-PCR) assays following screening by USDA-FSIS MLG 8.11.

Methods*:* Verification paired studies of 125g matrices consisting of 10 inoculated samples at ≤13 CFU and 5 uninoculated samples per matrix were conducted. Three matrices, RTE hotdogs, RTE breaded chicken nuggets and liquid whole eggs, were evaluated totaling 45 samples. Samples were screened and confirmed using the USDA-FSIS MLG 8.11. For colony PCR, 1-2 isolated colonies of *L. monocytogenes* from ALOA® (Agar Listeria Ottavani & Agosti) and MOX (Modified Oxford) agars were inoculated into 0.5 mL of Tryptone Salt Broth, lysed, and evaluated on GENE-UP *Listeria* (LIS) and *L. monocytogenes* (LMO) using the manufacturer's recommendations. Direct samples from primary and secondary enrichments (from MLG 8.11) were also analyzed on LIS and LMO assays. In total, at least 60 colonies were analyzed during this study.

Results*:* All uninoculated controls tested negative on target assays and reference confirmations with no growth on selective chromogenic agar. All inoculated samples correctly reported positive on LIS and LMO assays from primary and secondary enrichments and from colony PCR. Results from alternative and reference screening methods demonstrated 100% alignment with PCR results, colony confirmations and biochemical identifications.

Significance: This data supports real-time PCR colony confirmation as a rapid identification method for detecting *L. monocytogenes* pathogens in Liquid Whole Eggs and several RTE food products.

P2-185 Optimizing the Oxford Nanopore Technologies Flongle Flow Cell for Rapid Detection of Foodborne Pathogens in Whole Chicken Rinsate

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Introduction: Approximately 9.4 million cases of foodborne illnesses occur annually in the USA and hence the rapid detection of foodborne pathogens in meat and poultry products is vital to food safety.

Purpose: To optimize the ONT Flongle sequencing for the rapid detection of *E. coli* O15:H7, *Salmonella enterica, Listeria monocytogenes,* and *Campylobacter* species in whole chicken rinsate.

Methods: Whole chicken carcasses were rinsed with sterile 400 ml buffered peptone water, filter sterilized, and then spiked with various inoculum concentrations of each tested pathogen. A total of 30 ml of each inoculated chicken rinsate was mixed with 30 ml of the appropriate enrichment media for each pathogen and incubated at the appropriate conditions for up to 24 hours. All samples were processed in triplicates. For each spiked sample, 2 ml from each enrichment media were collected at 0, 6, 12, 18, and 24 hr and mixed for DNA isolation using Qiagen DNeasy Blood and Tissue Kit and then used for Native Barcoding library preparation and loaded on ONT Flongle flow cell.

Results: Up to 12 barcoded libraries were loaded in a single Flongle sequencing run which generated enough sequence reads within 24 hours using fast Basecalling to identify bacterial species when EPI2ME Fastq WIMP was run concurrently. Species level identification, with 1% minimum abundance cutoff value, was successful for all spiked samples. Using ONT flongle sequencing, the tested bacterial pathogens were identified as early as 6hr, 12hr, 24hr, and 24hr for *Salmonella* enterica, *E. coli* O157:H7, *Listeria monocytogenes*, and *Campylobacter* species respectively.

Significance: The success of ONT Flongle sequencing in accurately identifying multiple foodborne pathogens in chicken rinsate within 24 hours of enrichment and in a single sequencing run is very valuable and could serve as a rapid detection method replacing the laborious time-consuming conventional culture methods.

P2-186 Rapid Detection of *Salmonella* spp. Using the Loop-Mediated Isothermal Amplification (LAMP) Assay – Bioluminescent in Primary Production Boot Swabs Collected from Farms at Sanitary Void Moment

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Introduction: Brazil is one of the world's largest exporters of poultry products. To maintain safe production, sanitary measures such as the analysis of broiler litter for monitoring and controlling *Salmonella* spp. are necessary from farm to processor These highly alkalinized samples are collected by dragging moistened boot swabs in the environment. Choosing a rapid and accurate *Salmonella* spp. detection method in primary production samples is important for monitoring intervention effectiveness and for taking action to avoid product contamination.

Purpose: Determine the sensitivity, relative trueness (RT), relative limit of detection (RLOD), and acceptability limit (AL) of the *Salmonella* LAMP-Bioluminescent assay in primary production boot swabs compared to ISO 6579-1:2017.

Methods: Boot swabs samples (n=60) from a Brazilian middle east region farm, were analyzed in an unpaired testing comparing the rapid method to ISO 6579-1:2017 (n=30 each). Sample composition was broiler litter (e.g., soil, feathers, feces, wooden shavings, and lime powder) and sterile boot swabs moistened with BPW ISO (10 mL). Fractional artificial contamination was used, five were blank and the strain (*Salmonella* Typhimurium ATCC 14028, adapted to the matrix), was spiked in 2 levels, low (n=20, N1=0.2-2 CFU/test portion) and high (n=5, N2=5 CFU/test portion). Samples were enriched with BPW ISO (360mL for the rapid and 225 mL for the cultural methods), incubated (37°C/22-26 hours) and analyzed with LAMP-Bioluminescent assay and by ISO 6579-1:2017. Parameters required by ISO 16140-2:2016 for method comparison were determined.

Results: Blank samples presented all negative results and high population spiked samples presented all positive results for both methods compared. Low population spiked samples presented 16/20 positive agreements, 12 negative agreements and 2 negative deviations. The alternative LAMP-Bioluminescent presented sensitivity, RT, RLOD and AL of 89%, 93.3%, 1.00, being the rapid method fit for purpose.

Significance: The Neogen® Molecular Detection Assay 2 - Salmonella enabled reliable and rapid detection of *Salmonella* spp. in highly alkaline primary production alkaline sanitary void boot swabs.

P2-187 Oxidative Stability of Burger Containing Unconventional Food Plants and Packaged with a Novel Biodegradable Film Incorporated with Apple Pomace

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Introduction: Biodegradable films prepared from polybutylene adipate terephthalate (PBAT) and underutilized agro-industrial residues from apple juice extraction are alternatives to non-biodegradable packaging materials.

Purpose: The purpose of this study was to prepare a biodegradable plastic film extruded with PBAT, cassava starch and apple pomace and evaluate the oxidative stability, pH and water activity in burgers containing UFP such as dandelion (*Taraxacum officinale*) and ora-pro-nobis (*Pereskia aculeate*) packaged with these films.

Methods: The film was prepared with cassava starch, glycerol, PBAT and apple pomace by single-screw extruder (BGM Ind.,Taboão da Serra, SP, Brazil). The antioxidant activity of UFP was explored. Burger formulations containing ora-pro-nobis, dandelion and control were produced. The burgers were evaluated by thiobarbituric acid reactive substances (TBARS) assay during refrigerated storage (-2 °C). Malondialdehyde (MDA) production, pH and water activity were measured one day each week, starting from 6-day production until 30 days after. All experiments were performed in triplicate. Analysis of variance (ANOVA) was performed to analyze the data. Means were compared by Tukey's test and the results were considered statistically significant when $p < 0.05$.

Results: The antioxidant activity of dandelion was higher than ora-pro-nóbis. The lipid oxidation, it increased over time, on average, from 4.91±0.38 mg to 10.59±1.55 mg MDA/Kg meat. The burger control treatment suffered more intense oxidation than those with UFP. The lipid oxidation of the burger was significantly inhibited at 18 days of storage in the presence of ora-pro-nóbis (5.83±0.46 mg MDA/Kg meat) and dandelion (5.63±0.10 mg MDA/Kg meat) when compared to the control (9.07±0.50 mg MDA/Kg meat). The pH of burgers packaged with the film were time-dependent, while the water activity did not differ during storage.

Significance: These results indicate a strong antioxidative effect of ora-pro-nobis and dandelion in burger meat. This is probably due to the gradual release of the bioactive compounds from ora-pro-nobis, dandelion and biodegradable film enhanced with apple pomace.

P2-188 Effect of Vinegar and Natural Antioxidants on Shelf-life Enhancement of Turkey Deli Meat

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Introduction: With increased concern regarding synthetic preservatives, greater interest has been placed on use of natural preservatives. Use of vinegar and rosemary extract can help improve shelf-life of meat systems as well as give antioxidant protection.

Purpose: Evaluate impact of vinegar (IsoAge DV100) and rosemary extract on the microbial counts and sensorial attributes of deli turkey meat.

Methods: Deli-style turkey was manufactured and treated with, 0.7% IsoAge DV100 in combination with rosemary extract at concentrations of 40, 60, and 80 ppm for 60% carnosic acid delivery, 0.7% IsoAge DV100 alone (vinegar control), and no preservatives (control). Microanalysis treatments were inoculated (*L. monocytogenes*, *L. sakei*, or uninoculated), vacuum-packaged and stored at 4°C. Samples (duplicates), were removed from storage, homogenized, and plated onto selective media for enumeration. Failure of antilisterial capacity and spoilage threshold was assessed at 2 and 6 log CFU/g outgrowth, respectively. Treatment performances were compared using one-way ANOVA at $p<0.05$. Sensory was conducted, in parallel, at various points through days 0, 14, 28, 42, 70, and 96. Treatments were deemed unacceptable when 40% of panelists indicated it unacceptable.

Results: For *L. monocytogenes*, the control treatment exhibited fastest outgrowth >2 log CFU/g by 10 days of storage at 4°C. Treatment with 0.75% IsoAge DV100 and rosemary extract (>60 ppm) significantly ($p<0.05$) controlled *L. monocytogenes* outgrowth (<2 log CFU/g) for 78 days of storage . For spoilage bacteria (*L. sakei*) all treatments showed a significant ($p<0.05$) extension of 12 days over the control (spoiled at 30 days). Uninoculated samples stored at 4°C did not spoil until 150 days, ensuring low background. For sensory, IsoAge DV100 and 60ppm rosemary extract were considered acceptable through 96 days.

Significance: The study outlines the food safety and shelf-life aspect of vinegar-based solution. Additionally, significant antioxidant protection can be

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achieved by higher dosage of rosemary extract without compromising sensory aspect.

P2-189 Evaluating the Efficacy of Different Weak Organic Acid Salts against *Listeria monocytogenes* in an Uncured Turkey Deli Meat System

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Introduction: Sodium reduction and natural selection have increased potassium-based solutions as preferred antimicrobials. In the present work, potassium acetate/diacetate-blend is evaluated on antimicrobial efficacy against *L. monocytogenes* on deli meat systems.
Purpose: To assess the antilisterial effects of potassium acetate/diacetate (Provian K) in uncured turkey deli meat stored at 4 °C for 120 days.
Methods: A total of 300 pounds of deli turkey was formulated for different treatments (0.25-0.75% Provian K, and 1.25%-3.50% potassium lactate/sodium diacetate commercial blend) and control (no antimicrobials) were inoculated with five-strain cocktail of *L. monocytogenes*. Following inoculation, the turkey was vacuum packaged, and stored at 4°C sampled up to 120 days. Turkey samples (duplicates) were homogenized with a stomacher and plated onto Modified Oxford media for enumeration. Failure of antilisterial capacity was assessed at 2 log CFU/g outgrowth, and treatment performance was compared using one-way ANOVA (p<0.05).
Results: Inoculation level of 2 log CFU/g of *L. monocytogenes* was achieved on day 0 for all the treatments. The uncured control treatment exhibited the fastest outgrowth (>2 log CFU/g) of *L. monocytogenes* by day 21 of storage at 4°C. The highest concentrations of Provian K and potassium lactate/sodium diacetate commercial blend treatments-maintained control over *L. monocytogenes* outgrowth (<2 log CFU/g) and significantly extended the shelf life and food safety during storage (>42 days).
Significance: Potassium acetates/diacetates can be used as highly effective alternatives to classic lactate-based *Listeria* interventions in uncured deli meat.

P2-190 Evaluation of Dose-Response of Sodium Nitrite Concentration on *Listeria monocytogenes* in Frankfurters

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Introduction: Sodium Nitrite, used to prevent Clostridium spp. in processed meats, could additionally inhibit L. monocytogenes outgrowth. Due to varying usage-regulations across regions and food products, capturing dose response is essential.
Purpose: To evaluate the antimicrobial efficacy of sodium nitrite ($NaNO_2$) via dose response in a model frankfurter system.
Methods: Frankfurters were manufactured with chicken, pork, and 35% brine extensions resulting in 0-300 ppm sodium nitrite content. Base brine formulation included 2.0% salt (NaCl), 0.5% sodium phosphate, 1.79% seasoning (sodium erythorbate), 5.50% corn syrup, and 2.25% starch on total formula basis. Samples were inoculated with five-strain cocktail of *L. monocytogenes* (serotype 1/2a, 1/2b, 4a and 4b) at ca. 3 log CFU/g, vacuum-packaged, and stored at 4°C. Sampling (duplicates) were removed from storage, homogenized, and plated onto Modified Oxford medium for enumeration. Failure of antilisterial capacity and maximum population density post stationary phase was considered at 2 and 8 log CFU/g, respectively. Data generated was used for primary modeling using modified Gompertz equation to calculate maximum growth rate (μmax; log/day) and lag time (days). Differences among treatments were determined using one-way ANOVA at $P<0.05$.
Results: Overall, it was observed that with increasing dose of $NaNO_2$ in the formulation, there was an increase in days to 2 log CFU/g. Treatments with ≥120ppm $NaNO_2$ exhibited significantly ($p<0.05$) longer time to 2-log outgrowth after 33 days with longest being the 300-ppm $NaNO_2$ at 37 days compared to control and formulations with ≤60 ppm $NaNO_2$ at 12 days. It was also observed that with increasing dose of $NaNO_2$ in the formulation 80-300 ppm, there was a significant ($p<0.05$) decrease in the exponential growth rate and increase in the lag time (0.03-0.05 d^{-1}; 9-14 d) compared to formulations with 0-60 ppm $NaNO_2$ (0.10-0.18 d^{-1}; 2-5 d).
Significance: Data generated in this study can aid in decisions for secondary process necessitation such as adjusting dosage of natural curing agents in meat products.

P2-191 Enhancement of Fresh Ground Poultry Shelf-Life Using a Natural Vinegar and Plant Extract-Based Antimicrobial and Antioxidant Preservation System

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✦ ***Undergraduate Student Award Entrant***

Introduction: Fresh ground poultry shelf life is limited and consumer interest in natural and clean label preservation system and reducing food waste requires enhanced shelf life.
Purpose: To determine the antimicrobial efficacy of a vinegar and natural flavor solution against spoilage microorganisms in ground poultry.
Methods: Fresh ground chicken was formulated as (i) no preservative control (K), (ii) Natural Flavor at 0.006% (L), and (iii) 0.756% vinegar-natural flavor(M). Treatments were stored at 4°C for up to 25 days; their efficacy was assessed against Lactic Acid Bacteria (LAB), Aerobic Plate Count (APC), and Enterobacteriaceae (EB) for up to 25 days. Spoilage threshold and was set at 6 log CFU/g. Primary modelling with modified Gompertz equation was used to calculate days to reach spoilage threshold for each treatment. Additionally, the oxidation by-product: malondialdehyde (MDA) in each treatment was determined via Thiobarbituric Acid Reactive Substances (TBARS) assay at day 0 and 25. Statistical analysis and model building was performed in JMP Pro version 16.1.0 (SAS Institute Inc., NC, USA), at $p < 0.05$.
***Results*:** Overall, treatment M demonstrated superior efficacy in controlling growth of spoilage microorganisms compared to other treatments. Specifically, for LAB, APC, and EB levels, treatment M showed a significant increase ($p < 0.05$) in shelf-life by 5, 6, and 10 days, respectively, as compared to the no preservative control which reached the spoilage threshold by day 7, 6, and 5, respectively. Treatment L reached spoilage threshold (6 logs CFU/g) at 7 days of refrigerated storage. Treatment M also exhibited a significantly lower change (0.95μg/g) in the MDA value from start to end of shelf-life as compared to the no-preservative control (3.00 μg/g).
Significance: The combination of vinegar and natural flavor demonstrates promising antimicrobial as well as antioxidant functionality, contributing to extensively enhancing the shelf-life and quality of ground poultry while meeting consumer preferences for clean label meat products.

P2-192 Effect of Thawing Techniques on the Loads of Microbial and Spoilage Indicators in Chicken Tenders during Distribution in the Food Service Value Chain

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Introduction: Poultry meat is the most popular animal protein globally. Inadequate product handling during distribution and thawing can contribute to spoilage and food waste.
Objective: Determine the dynamics of microbial and spoilage indicators at different stages in the distribution chain by applying two different thawing

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methods for chicken tenders stored under different freezing methods, as compared with product stored under refrigerated conditions.

Methods: A microbiological study to evaluate the shelf-life of chicken tenders in eleven sampling points in the distribution value chain was conducted. Freshly processed chicken tenders were procured from a commercial processor in the Southeast region of the US and shipped under refrigerated conditions. 30 tenders were stored at different conditions: fridge (4 °C), freezer (-18°C) and blast freezer (-40°C) temperatures. 5 replicates of each condition were thawing following 2 methods, fridge (24 hrs to 4 °C) and counter (6 hrs in room temperature). Tempo System protocol was performed to quantify counts of aerobic total counts, Enterobacteriaceae, and psychrotrophs.

Results: For APC, it was observed the counter method (6h) and the fridge method (24h) exhibited a decrease in contamination from 3.1 logCFU/g (Initial count) to 1.93 log CFU/g and 2.6 log CFU/g respectively. Enterobacteriaceae under blast freezer storage, a decrease in the bacterial count was recorded between the initial count (2.7 log CFU/g), the counter-thawing method (1.5 log CFU/g) and the fridge method (<0.5 log CFU/g). No significant differences were found between both thawing methods in the three microbial indicators (p>0.05). Initial counts for psychrotrophs (7.87 logCFU/g) were equals (p>0.05) compared with counter (7.1 log CFU/g) and fridge (7.2 log CFU/g) methods.

Significance: Identifying the most efficient methods for thawing chicken-meat and preventing microbial growth could be a significant advance in food safety and can contribute to reductions in food waste. A detailed understanding of the microbial dynamics of microbial and spoilage indicators could help processors and food service operators.

P2-193 Shelf-Life Extension of Poultry Using Zero-Oxtech® Packaging System: Minimizing Food Safety Issues and Maximizing Poultry Process Operations

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Introduction: Zero-OxTech® process guaranteeing extended shelf-life in an absolute zero-oxygen atmosphere is based on the concept to preserve MRA [METMYOGLOBIN REDUCING ACTIVITY] natural enzymes within the muscle.

Purpose: The objective was to access the efficacy of Zero-OxTech® process to extend the conventional 14-day shelf-life of poultry meat to maximize efficacy of poultry plant manufacturing operations for domestic transportation and distribution.

Methods: Small birds were collected from local poultry plant in Ontario. Birds were placed into one of two treatments. The first treatment was the control group, which was packaged using the current open nozzle packaging system with the addition of a high barrier (HB) bag. The second treatment used current equipment using a Zero-OxTech® HB bag, Zero-OxTech® CO_2 generator and Zero-OxTech® sachets. Both treatment samples were stored at 4C for up to 28 days and tested for aerobic plate counts (APC), lactic acid bacteria, and anaerobic plate counts on day 2, 14, 16, 19, 21, 23, 26, and 28. The experiments were repeated once, and each trial was performed with duplicate samples. Trained panelists were used for sensory evaluation.

Results: The Zero-OxTech® process provided a significant increase (p<0.001) in shelf-life to poultry meat from conventional 14 days to 28 days. The APC in Zero-OxTech® HB packaged samples reached 5 logs at day 19. Zero-OxTech® process significantly inhibited (p<0.001) growth of aerobic bacteria with population densities <4.5 logs at day 28. As with APC, anaerobic bacteria counts were consistently lowered in Zero-OxTech® process as compared to the conventional packaged samples throughout the 28 d testing period. Lactic acid bacteria counts increase was non-significant (p<0.001) in case of Zero-OxTech treated samples. Also, these meat samples were found to be organoleptically acceptable.

Significance: Zero-OxTech® packaging system was capable to extend the shelf-life of the poultry meat.

P2-194 Evaluation of Bacteria from Swine-Related Sources as Direct-Fed Probiotics for Enhancement of Feed Utilization and Growth Performance in Swine

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❖ ***Developing Scientist Entrant***

Introduction: The gastrointestinal tract of swine harbors a significant number of bacteria for digestion of feed, nutrient absorption, and growth. Enhancing feed digestion in animals could lead to improvements in weight gain and profitability. Consumer groups and regulatory agencies focus on limiting the use of antibiotics as growth promotants to address global health crisis from multi-drug resistant bacteria. Bacterial probiotics with enzymatic activities provide a desirable alternative for enhancing digestibility, growth, and profitability.

Purpose: To identify the beneficial biochemical properties of swine-related intestinal bacteria for potential use in the development of probiotic cultures for pigs.

Methods: Bacteria from the small intestine and feces of freshly slaughtered swine were screened *in vitro* by direct plating on differential agars for detection of proteolytic, saccharolytic, lipolytic, and cellulolytic metabolic activities and incubated anaerobically at 37°C for 48-72 hours. Bacterial colonies showing positive bioassay results were reconfirmed and their identities verified by 16S rRNA PCR, sequencing, and BLAST search of NCBI databases. Cultures were prepared for *in vivo* pig trials at the OSU Swine Research Facility involving 280 nursery pigs that were randomly assigned to one of four treatment groups: (a) control, (b) probiotics in water, (c) probiotics in feed, and (d) probiotics in feed and water.

Results: Isolates showing positive bioassays for various metabolic activities were identified as *Bifidobacterium porcinum*, *Bifidobacterium thermophilum*, *Streptococcus lutetiensis and Limosilactobacillus reuteri*. Among the treatment groups receiving probiotic cultures, the one in which probiotics were only added to water demonstrated a higher average weight gain compared to the control group suggesting potential to improve swine weight gain and enhance feed efficiency.

Significance: Using biochemically active cultures to promote feed digestion can improve the animal's nutrient absorption and utilization. This research has the potential to enhance swine weight gain and feed efficiency, thus improving swine productivity and profitability.

P2-195 Mapping of Stakeholders Involved in Avian Influenza Surveillance in Canada

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Introduction: During avian influenza (AI) outbreaks, decisions concerning public and animal health must be made rapidly to minimize spread and mortality among poultry; however, decision makers lack systems that aid in use of surveillance data and other evidence to support their decisions.

Purpose: The aim of this project was to identify stakeholders and their roles in AI surveillance in Canada to support the development and optimization of a decision support system to aid with AI detection and control in Canada.

Methods: An initial list of stakeholders was developed by subject matter experts at the University of Guelph. Additional stakeholders were identified using targeted web searches and a snowball approach until no new stakeholders were identified. Public contact information was then retrieved, and stakeholders were invited to refine the stakeholder list. Stakeholders were then mapped by levels of organization (international, national, provincial/territorial) and their roles in surveillance categorized.

Results: Over a 3-week period, 34 responses were received from 55 initial stakeholders contacted (response rate = 62%). The final stakeholder list included 229 stakeholders (7 international, 60 national, 162 provincial/territorial). Stakeholder roles included conducting research, managing reporting hotlines, maintaining poultry supply, promotion of sustainable agriculture, protecting animal health, monitoring human and animal cases of AI, and providing diagnostic services.

Significance: The stakeholder map identifies and characterizes stakeholders involved in complex responses to AI outbreaks and who may benefit from a decision support system used to inform future outbreak responses in Canada. Effective decision support systems are ones designed with feedback from stakeholders; having a list of stakeholders allows for easy identification of who to involve in future projects to aid in development and optimization of a decision support system.

P2-196 Consumer Perceptions of Meat and Poultry Safety at Kentucky Farmers' Markets

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Introduction: With the growing demand for "local" foods, farmers' markets are increasingly becoming a significant and integral part of the local food system.

Purpose: This study aimed to assess consumer perceptions of locally processed meat and poultry sold at farmers' markets.

Methods: Face-to-face surveys were conducted with 314 consumers at 12 farmers' markets spread across Kentucky. Statistical analyses, including descriptive statistics, frequency distribution, cross-tabulation, and Chi-square tests of independence, were employed.

Results: Findings revealed that 29.9% of participants had previously purchased meat, and 16% purchased poultry from local farmers' markets 1-3 times a season. Nearly 78% of participants agreed or strongly agreed that meat and poultry products at farmers' markets are safe. However, only 47.9% believed that locally produced meat and poultry are safer than conventional options at commercial supermarkets. Consumer opinions on pathogenic bacteria presence on poultry at farmers' markets were divided, with 33.1% agreeing and 34.4% disagreeing. Half of the participants support government regulation of meat and poultry products at Kentucky farmers' markets, while 21% do not. Regarding additional food safety interventions at farmers' markets, 46.3% agreed or strongly agreed, while only 13.8% disagreed or strongly disagreed.

Significance: Understanding consumer perceptions enables stakeholders, including growers and vendors, to make well-informed decisions about food safety policies. These findings assist stakeholders in determining effective food safety interventions for enhancing the safety of meat and poultry products at farmers' markets.

P2-197 Growth Potential of *Clostridium perfringens* during Cooling of Large Mass, Non-Intact Beef Products

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Introduction: FSIS Appendix B has been revised in December 2021 with new available cooling options. FSIS suggests performing challenge studies or process validations for large mass, non-intact products that cannot chill quickly to fill the scientific gap for those products.

Purpose: Objective of this study was to investigate *Clostridium perfringens* growth in large mass, non-intact RTE beef products during extended cooling conditions.

Methods: Seven raw beef formula samples with sizes larger than 4.5 inches or weight of 8 pounds (n=4 per treatment, 22 g) were inoculated with a 3-strain *C. perfringens* spore cocktail for a 10^3 CFU/g final population. Samples with and without nitrite and antimicrobials were vacuum-packaged and submerged in a circulating water bath, and then heated to 71.1°C for 20 min to heat shock the spores and kill vegetative cells. Subsequently, samples were chilled continuously per Appendix B-Option 1.5 with extended 1st stage from 54.4 to 26.7°C within ≤2.5 or 3 hours, and then from 26.7 to 4.4°C within ≤5 hours. After each processing step, *C. perfringens* counts were enumerated by direct plating on TSC agar without egg yolk.

Results: Pot roast samples with antimicrobial had significant *C. perfringens* outgrowth after 1st chill cycle (54.4 to 26.7°C in 3 hours), however, growth was <1 log at the end of the process. Samples which contain antimicrobials including lactate/diacetate and vinegars and follow the chilling process from 54.4 to 26.7°C within 2.5 hours and from 26.7 to 4.4°C within 5 hours (total 7.5 hours) demonstrated the most ideal and safest chilling under FSIS Appendix B. Samples formulated without nitrite allowed slightly higher *C. perfringens* outgrowth than the samples formulated with nitrite($P>0.05$).

Significance: Challenge studies provide scientific support and guidance on chilling processes of large mass products that cannot chill quickly enough to follow the new options in revised Appendix B.

P2-198 FSIS Market Basket Study Results for *C. perfringens* in Large Mass Ready-to-Eat Products

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Introduction: The Food Safety and Inspection Service (FSIS) commissioned a study through the Food Emergency Response Network (FERN) laboratories to assess levels of *Clostridium perfringens* (*C. perfringens*) in certain federally inspected ready-to-eat (RTE) meat and poultry products sold at retail locations.

Purpose: The study aimed to determine the potential risk to consumers from large mass non-intact products such as injected turkey breast or roast beef.

Methods: The FERN laboratories purchased RTE beef, poultry, and pork shoulder products sold at retail locations in eight states (California, Colorado, Iowa, Michigan, Minnesota, Missouri, Ohio, and Virginia). Large mass (> 4 inches in diameter or > 8 lbs.) non-intact products that were formulated without antimicrobial agents (e.g., nitrite, including from natural sources) were tested. These products were selected because they require an extended cooling time, which could allow *C. perfringens* spore germination and outgrowth. Samples were analyzed for *C. perfringens* spores using a modified method adding an additional heating step to kill vegetative cells, so that the colonies, when plated, represented any spores in the sample.

Results: In total, 494 products were collected and tested in eight states. One RTE beef sample had a low level of *C. perfringens* at 1.08 log CFU/g (12 CFU/g). All other products tested were below the limit of detection (0.5 log CFU/g). The one positive result was below the level of public health concern because > 6 log of *C. perfringens* is needed for illness to occur.

Significance: The results of the study demonstrate that the risk to following the cooling parameters for these products in FSIS cooling guidance (Revised Appendix B) is minimal.

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P2-199 Genomic Analysis of *Clostridium perfringens* from Broilers Raised Conventionally and Without Antibiotics

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Introduction: *Clostridium perfringens* is a zoonotic pathogen associated with foodborne illness in humans and necrotic enteritis in poultry, often controlled with antimicrobials in conventionally raised broiler flocks; however, the pathogen is re-emerging as limitations are placed on the use of antimicrobials as growth promoters.

Purpose: To investigate the genomic associations of virulence genes harbored by *C. perfringens* from broilers raised conventionally and without antibiotics to clinical *C. perfringens* poultry isolates.

Methods: *C. perfringens* strains (n=123) were isolated from broilers at intervals (hatch to transportation to processing plant) during production, over two seasons from two types of farms (conventional and NAE). Genomes were sequenced using long-read native DNA sequencing. Contaminant reads were removed with Kaiju v1.10.0. Assembly was completed with Bactopia v3.0.0. Scaffolds were generated using ARCS v1.2.6, followed by analysis in Roary v3.13.0 and Scoary v1.6.16 to generate a pangenome and evaluate associations between genes and traits, respectively. Virulence and antimicrobial resistance gene annotation was completed *in silico* with amrFinderPlus v3.11.26. Publicly available genomes of *C. perfringens* isolates (n=28) from diseased chickens were screened with GUNC v1.0.6 to evaluate assembly completeness and quality and remove non-*Clostridium* contaminant contigs.

Results: The prevalence of *C. perfringens* was higher (44.1%) in NAE broilers compared with conventional (35.52%; p=0.008) and higher in summer (58.3%) than fall (21.3%, p<0.001). Instances of nearly identical strains from healthy birds on different farms during different seasons but within a production system may indicate horizontal transmission of the pathogen. Limited genetic diversity of strains from healthy and diseased birds separated by time and geography emphasizes the importance of gene acquisition in pathogenesis.

Significance: The growing consumer preference for poultry meat raised without antibiotics necessitates a better understanding of how reduction in AGP use affects virulence of *C. perfringens* strains present within the food system.

P2-200 Development of a Long-Read, Native DNA Sequencing Analysis Pipeline Using a Curated *Salmonella enterica* subsp. *enterica* Database

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Introduction: Advances in next-generation sequencing offer potential for high-resolution analysis and characterization of microbial populations in food-related matrices to better inform food safety-related decision making.
Purpose: To develop a protocol utilizing a custom database and optimized metagenomic analysis pipeline parameters for long-read, shotgun metagenomic sequencing of native microbial DNA from poultry matrices for high-resolution characterization of *Salmonella enterica* subsp. *enterica.*
Methods: Sequencing was carried out with the Oxford Nanopore Minion with R10.4.1 flow cells, the rapid barcoding 96 V14, and native barcoding 96 V14 library preparation kits. Samples (n=80) were derived from raw poultry rinses (n=10), primary enrichments (n=10), secondary enrichments in three selective broths (n=10 per broth; n=30), and colonies from selective agar plates (n=30). Basecalling in Dorado used the fast, high accuracy, and super-accurate models. Reads were analyzed using taxprofiler v1.1.3 pipeline with the classification and profiling packages kaiju v1.10.0, centrifuge v1.0.4, and krakenuniq v1.0.4. A custom *Salmonella enterica* subsp. *enterica* genome set was compiled from publicly available genomes subjected to filtering and cleaning for quality and contaminant removal, followed by *in silico* characterization of the remaining contigs. This sequence set was used to construct package-specific databases.
Results: Native barcoding V14 kit produced mean Phred quality scores of 18, 33, and 39 for the fast, high accuracy, and super-accurate models, respectively, compared to Phred scores of 15, 23, and 28 for the rapid barcoding V14 kit, values approximately equivalent to a 10-fold difference in per-base accuracy for the super-accurate model. The native barcoding kit produced mean read lengths ~100% longer than the rapid barcoding kit (<2000 bp compared to ~4000 bp), which is not unexpected because rapid barcoding involves cleavage of DNA fragments.
Significance: Understanding limits and capabilities of next-generation sequencing and how analysis parameters affect outcomes is critical to proper implementation of such technology into food safety.

P2-201 Effects of Feed Additives on Production Parameters and Cecal Microbiota of Late-Laying Hens

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Introduction: Antibiotic growth promotors (AGP) have widely been used in non-therapeutic dosages to improve livestock production and health. However, it is necessary to explore feed additives that exhibit similar properties, without the continued usage of anti-microbials. One alternative being examined is the usage of essential oils (EO), and their impact on production parameters and gut health.
Purpose: This research examines the feasibility of EO and prebiotic application in feed as an AGP substitution and its impact on production parameters and cecal microbiota.
Methods: Fifteen laying hens were randomly assigned to each treatment group (n=60) consisting of – 1) corn-soybean meal-based basal diet, 2) 0.5% low EO, 3) 1% high EO, and 4) 1% prebiotic mixed diets. The addition of EO and a prebiotics diet lasted for 12 weeks. Egg production was determined by eggs collected daily with an expected egg output of 7 eggs per week, egg quality was measured bi-weekly, and body mass was measured monthly. This project complies with all IACUC standards. Data was analyzed using repeated measures ANOVA in SAS 9.4. For cecal microbiota, the primers (341F/805R) were designed to target the V3 and V4 regions of 16S rDNA from cecal contents and the amplified library is sequenced on a NovaSeq platform.
Results: Results showed that body mass was affected significantly ($p < 0.05$) by the prebiotic diet when compared with the control diet-fed hens. Likewise, prebiotic-fed hens have significantly lower ($p < 0.05$) feed intake values when compared with the rest of the groups. The relative abundance of cecal microbiota showed > 20% of Lactobacillales order. However, the presence of most of the Lactobacillales were significantly higher ($p < 0.05$) in cecal contents when compared to ileum.
Significance: EO supplementation could prove beneficial to reducing antibiotic usage, which could further help impede the emergence of antibiotic-resistant pathogens.

P2-202 Advancing Antimicrobial Selection: An Advanced Predictive Model for *Listeria monocytogenes* in Industrial Food Production

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Introduction: Mathematical models serve as valuable tools for quick evaluation and selection of antimicrobials in the development and reformulation of ready-to-eat meat products. Predictive models are increasingly gaining acceptance in the food industry to enhance food safety through risk assessment.
Purpose*:* Developing food-specific models is challenging and impractical, broth-based models offer conservative predictions in food matrices. This study aimed to collect optical-density data in Bioscreen for *Listeria monocytogenes* and develop models for growth and lag time prediction.
Methods*:* This research involves the collection of large datasets, transforming data through calibration, estimating growth parameters, and developing predictive models. A total of 9400 optical density (OD) curves were generated in duplicates under variable conditions (temperature: 7-37°C, pH: 4.5-7.5, NaCl: 0-30%, antimicrobial-1 0-6%, antimicrobial-2: 0-6%, and antimicrobial-3: 0-5%). The antimicrobial solutions are weak organic acids produced by Kerry Inc. Additionally, about 299 growth rate data were collected from ComBase under variable conditions (temperature: 5-35°C, pH: 5-7.5, water activity: 0.97-0.997, and nitrite: 15-200 ppm). The Baranyi model was fitted to natural log-transformed data to estimate growth rate and ho values. Calibration experiments were conducted to transform growth rates from ln(OD/h) to ln(CFU/h). The physiological state (ho's) between OD and CFU curves was also calibrated and then used to predict the lag time.
Results*:* A Gamma model was constructed to analyze the effect of seven gamma factors on *L. monocytogenes* growth. The developed gamma concept model includes an RMSE of 0.081 ln(CFU/h) and R^2 value of 0.893.
Significance: The model will be validated in food matrices data (literature+ experimental data) before its deployment for the selection of Kerry antimicrobials for industrial food manufacturing. Given that this model was built from large experimental datasets, it serves as a robust tool to predict *Listeria* behavior, thereby enhancing the shelf life of products and ensuring consumer safety.

P2-203 Effects of Liquid Smoke Treatment and Inoculation Levels on the Growth of *Listeria monocytogenes* in Broth

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Introduction: Understanding the growth of *Listeria monocytogenes* is crucial for devising effective control strategies.
Purpose: This study investigates the impact of inoculation level and a liquid smoke extract based preservative ingredient on the growth dynamics of *L. monocytogenes* in broth environments.
Methods: Eight strains of *Listeria monocytogenes* were tested in BHI broth at 20°C at different levels of liquid smoke (Cloud S-C100) and different inocu-

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lation levels. Liquid smoke levels ranged from 0-0.4% in increments of 0.1% and inoculation levels were 1-3-7.3 log CFU/ml with intervals of 2 log. Growth curves were generated using absorbance measurements (600nm), maximum growth rates (μmax; h-1) and lag time (h) were calculated using the modified Gompertz equation to determine MIC and analyzed using one-way ANOVA (p<0.05). If no growth was observed lag time was forced to last measuring point, which was 16 days.

***Results*:** The difference in inoculation level did not show any influence on the maximum growth rates of all eight *Listeria* strains. However, at lower Inoculation levels, the lag time was significantly longer (p<0.05). In addition, concentration of smoke had a significant impact on the growth of *L. monocytogenes*. Increase in concentration of Cloud S-C100 significantly increased the lag time (0% 1.1-1.2, 0.1% 1.5-1.8, 0.2% 2.9-4.3, 0.3% 4.0-16 and 0.4% 16 days) and reduced the growth rates (0% 2.6-3.8, 0.1% 2.0-3.2, 0.2% 1.0-2.3, 0.3% 0-2.4 and 0.4% 0 umax/h) for all tested *Listeria* strains. Cloud S-C100 at 0.4% did not show growth of all tested *Listeria* strains.

Significance: Cloud S-C100 has shown clear growth controlling properties for *Listeria monocytogenes* and could be proposed to be tested as a preservative in foods to control growth of *Listeria monocytogenes*.

P2-204 Effect of pH and Temperature on the Inactivation of Foodborne Pathogens in Cold Brew Coffee

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❖ ***Developing Scientist Entrant***

Introduction: Cold brew coffee, known for its distinct flavor and reduced acidity, was subjected to a 30-day storage period to assess its susceptibility to microbial growth, aiming to enhance our understanding of this popular beverage's safety.

Purpose: This study investigated the impact of pH levels (4.9 and 5.3) and storage temperatures (4°C and 23°C) on *Salmonella spp., Escherichia coli (E. coli),* and *Listeria monocytogenes* in cold brew coffee, unraveling pathogen-specific responses.

Methods: Filtered sterilized cold brew coffee was adjusted to pH 4.9 and 5.3, inoculated with *Salmonella enterica s.v* Typhimurium (ATCC K1028)*, Escherichia coli* O157:H7 B6-, and *Listeria monocytogenes* 10403S, and stored at 4°C and 23°C. Samples were collected, plated on selective media, and enumerated. Experiments were conducted in triplicates, monitoring pathogen-specific responses and employing parameter estimation techniques.

Results: *Salmonella* and *E. coli* exhibited pH-dependent survival trends. At pH 4.9, *Salmonella* reduced by 5 log CFU/ml after 7 days, reaching the limit of detection in 11 days. Conversely, at pH 5.3, a similar reduction occurred after 14 days, taking 25 days to reach the limit of detection level. Temperature played a crucial role, with *Salmonella* showing elevated survival risks at 23°C. *E. coli* displayed comparable trends. *Listeria* showcased resilience to pH variations at both temperatures. Mathematical modeling unveiled pH-specific trends: *Salmonella* favored log-linear modeling at pH 4.9 and Weibull modeling at pH 5.3, *E. coli* preferred log-linear in both scenarios, and *Listeria* favored log-linear at pH 4.9 and Weibull at pH 5.3. Lower temperatures prolonged survival, while higher temperatures accelerated reduction, especially notable in *Salmonella* and *E. coli.*

Significance: This research underscores the intricate microbial dynamics in cold brew coffee, emphasizing the importance of pH and temperature control. The findings provide valuable insights for the food industry to bolster safety measures through targeted interventions.

P2-205 Antimicrobial Effect of Bovine Lactoferrin and Glycerol Monolaurate on Selected Gram-Positive and Gram-Negative Pathogenic Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: Infant formula manufacturers aim to replicate the nutritional composition of mother's milk, incorporating essential immune-supporting elements. Lactoferrin and Glycerol Monolaurate (GML), crucial antibacterial compounds in human milk, hold the potential to enhance the formula's functionality by mimicking these essential components.

Purpose: This study investigates the antimicrobial efficacy of bovine lactoferrin (bLf) and Glycerol Monolaurate (GML) against pathogenic bacteria, targeting specific Gram-negative *(Cronobacter sakazakii* strains 12868 and 29004, *Pseudomonas aeruginosa*, and *Salmonella enterica* Typhimurium) and Gram-positive (Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Listeria monocytogenes*) strains.

Methods: Bactericidal properties of bLf and GML were assessed through Agar diffusion assays, Minimal Bactericidal Concentration (MBC) analysis and challenge studies of Infant formula (PIF) fortified with bLf and GML alone and in combination with selected pathogens.

Results: bLf at 16mg/disc strongly inhibited all strains except *S. enterica*, while GML at 8mg/disc exhibited strong inhibition across all strains. Gram-positive bacteria showed susceptibility to GML, with strong inhibition achieved at 5mg/disc. MBC analysis aimed for ≥3 log reduction, revealing higher susceptibility of *S. Typhimurium* and *L. monocytogenes* to bLf (87.2 & 59.52μM, respectively) compared to other strains. GML exhibited higher susceptibility in MRSA and *L. monocytogenes* (6 & 10mg/ml, respectively) than Gram-negative strains (16-18mg/ml). Reconstituted infant formula, fortified with bLf, GML, bLf + GML, or Control at identified MBCs, was challenged with a high bacterial inoculum. Both GML and bLf individually resulted in significant log reduction at various time points. Within 72h, bLf + GML exhibited ≥3 log reduction for all bacterial strains and ≥6 log reduction for Gram-positive strains, surpassing the log reduction achieved by each component.

Significance: These findings underscore the efficacy of combining GML and bLf against specific environmental pathogens, suggesting potential applications in infant nutrition, safety, product spoilage prevention, and healthcare.

P2-206 Quantitative Microbial Spoilage Risk Assessment of *Aspergillus* Niger in White Bread Supply Chain

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Introduction: Food spoilage caused by post-baking contamination with mold existing in the industrial environment is a major concern in the bakery industry.

Purpose: This study aims to create an effective risk assessment model for managing *Aspergillus niger* contamination, which could support decision-making and improve control measures within the bakery supply chain.

Methods: A supply chain of white bread was examined, consisting of a processing plant and two retail stores. Detailed interviews were carried out to gather essential information about these businesses. To collect time-temperature profiles at each processing step, on-site visits were carried out during summer and winter using temperature data loggers. Predicted visual mycelium diameter derived from exposure modelling was validated by using a time-lapse camera. Additionally, six different "what-if" scenarios were proposed and evaluated.

Results: The mean risks of *A. niger* contamination per package sold at retailer A were 0.031 in summer and 0.027 in winter, while at retailer B were 0.016 in summer and 0.013 in winter. Sensitivity analyses revealed that retail storage time, retail temperature, and prevalence during cooling in the factory were the primary factors influencing the spoilage of white bread. "What-if" scenarios suggested that if current retail environmental temperatures were reduced by 1°C in both summer (from 23.97°C to 22.97°C) and winter (from 23.28°C to 22.28°C), spoilage risk could decrease significantly.

Significance: These findings underscore the importance of establishing a quantitative microbial risk assessment model for evaluating microbial spoilage in food products. With the application of such models using real data, food companies can make informed decisions about controlling measures to mitigate spoilage risks, prevent food waste, and thereby contribute to environmental sustainability.

P2-207 Predictive Model for Growth of *Salmonella*

Infantis in Ground Turkey during Temperature Abuse

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Introduction: Most samples (25 g) of ground turkey (GT) examined had no (74%) or low (< 1 log) levels of *Salmonella*. However, temperature abuse of GT can result in rapid growth and consumption of high levels of *Salmonella* and foodborne illness.

Purpose: To develop a model for predicting growth of *Salmonella* in GT during temperature abuse.

Methods: A GT isolate of *Salmonella* Infantis (0.84 log) was inoculated into GT samples (0.2 g), which were then incubated for 0 to 28 h at 16 to 44°C. A most probable number (MPN) method with a sensitivity of 0 log per sample was used to acquire growth data, which were fitted to a three-phase linear model (TPLM) to obtain data for lag time, growth rate, maximum population density, and 95% prediction interval (PI). Multiple layer feedforward neural networks (MLFNNs) were used to predict TPLM parameters as a function of temperature. A tertiary TPLM with embedded MLFNNs for TPLM parameters was developed and used to predict variability and uncertainty of *Salmonella* Infantis growth in GT as a function of time and temperature using 95% PIs. Predictions of the tertiary TPLM were compared to observed MPN data using criteria of the Acceptable Prediction Zones (APZ) method.

Results: The overall proportion of residuals in the APZ (pAPZ) was 0.8 for dependent data (n=468) and 0.84 for independent data (n=204). However, there were local prediction problems (pAPZ < 0.7) that limited the range of model validation to temperatures from 20 to 40°C where pAPZ was 0.84 for dependent data and 0.87 for independent data.

Significance: Within the validated temperature range, the model can be used with confidence to predict growth of *Salmonella* Infantis in GT during temperature abuse. The model fills an important data gap in risk assessments for *Salmonella* and GT.

P2-208 How Does Protein Concentration in Food Affect Bacterial Growth Kinetics? Development of Predictive Models for *Escherichia coli* Growth as a Function of Protein Concentration

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Introduction: Predictive models for bacterial growth and/or inactivation based on culture media data often illustrate different prediction from observation in foods. Although the differences would be due to the difference in the nutrients, its phase, and etc., the impact of those differences on the bacterial behavior has not been fully quantified so far.

***Purpose*:** This study aims to quantify the effects of nutrient, especially protein on the bacterial growth behavior and develop an alternative predictive growth model to fill the gap between the prediction by culture media-based model and observation in foods.

***Methods*:** Pre-grown *Escherichia coli* ATCC 25922 were inoculated in model matrices comprising soy protein (0 - 30%) and sterile pure water and the growth was evaluated at 37°C for up to 12 h. The observed growth kinetics data were analyzed by Baranyi-Roberts model and obtained maximum specific growth rate (μ_{max}) and lag time (λ). The secondary model for μ_{max} and natural logarithm of λ was described as a function of protein concentration by using the expanded square-root model by Ross. The developed models were validated by *E. coli* growth data in foods from ComBase database. The accuracy of the developed models was evaluated by root mean squared error (RMSE).

***Results*:** The secondary model considering protein concentration for μ_{max} of *E. coli* was successfully described as a function including natural logarithm of protein concentration. The RMSE of the μ_{max} model with and without protein concentration was 0.33 (1/h) in and 0.45 (1/h), respectively, indicating significant improvement of the model accuracy. The model for natural logarithm of λ with protein concentration also demonstrated slightly higher accuracy (RMSE = 0.98) than those without protein concentration model (RMSE = 1.09).

Significance: Considering protein concentrations will be effective to predict *E. coli* growth behavior in foods more accurately.

P2-209 Increased Thermal Resistance of *Escherichia coli* O157:H7 and *Salmonella* in Animal Fat – One-Step Kinetic Analysis

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Introduction: It is generally believed that animal fat could increase the thermal resistance of foodborne pathogens, such as *Escherichia coli* O157:H7 and *Salmonella*. However, quantitative data are scarce concerning thermal inactivation kinetics of foodborne pathogens in the presence of animal fat.

Objective: This study was conducted to measure the thermal resistance of *E. coli* O157:H7 and *Salmonella* in animal fat and determine the inactivation kinetics.

Methods: A 4-strain cocktail of *E. coli* O157:H7 or *Salmonella* was inoculated to tallow and subjected to heating at 55, 60, 65, 70, or 75 ℃ to observe bacterial survival. The survival curves of each microorganism were analyzed with the USDA IPMP-Global Fit to estimate the kinetic parameters and evaluate thermal resistance.

Results: The survival curves of microorganisms showed that the thermal inactivation did not follow the first-order kinetics and were nonlinear, upwardly concaved, with rate of inactivation decreasing with time, suggesting gradually increased resistance to heat under each treatment temperature. Such curves were analyzed with the Weibull model, yielding a < 1 exponent (0.362 for *E.* coli O157:H7 and 0.282 for *Salmonella*). The first decimal reduction time was 0.974, 0.247, 0.112, 0.075, and 0.05 min for *E. coli* O157:H7 and 0.184, 0.058, 0.030, 0.016, and 0.014 min for *Salmonella* at 55, 60, 65, 70, or 75 ℃, respectively, suggesting that *E. coli* O157:H7 and *Salmonella* could resist heating for longer time at higher temperatures in tallow than they do in regular meats containing lower levels of fat.

Significance: The results of kinetic analysis indicate that fat could significantly increase thermal resistance of *E. coli* O157:H7 and *Salmonella* and may be used to evaluate the safety of cooked beef patties and mechanically tenderized beef containing high levels of fat. More study is needed to examine the survival of foodborne pathogens in high fat products.

P2-210 Effect of Sodium Nitrite, Sodium Erythorbate, Sodium Tripolyphosphate, and Sodium Chloride on Inhibition of *Clostridium perfringens* in Cured Meat: Logistic Modeling and Development of Critical Control Surfaces

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Introduction: The growth of *Clostridium perfringens* in cooked meat and poultry products may cause food poisoning due to formation of *C. perfringens enterotoxin.*

Objective: This study was conducted to evaluate the effect of sodium nitrite ($NaNO_2$), sodium erythorbate (SE), sodium tripolyphosphate (STPP), and salt on growth of *C. perfringens* in cooked meat and to develop a no-growth boundary or critical control surfaces (CCS) to prevent its growth.

Methods: Shahidi Ferguson Perfringens agar (SFPA), inoculated with a 3-strain spore cocktail of *C. perfringens* with a combination of $NaNO_2$ (100-200 ppm), SE (0-547 ppm), STPP (0-0.5%), and salt (2-3%), was dispersed into 96-well microplates and incubated anaerobically an incubator programed to remain at 4°C for 24 h, heat to 80°C in 1.75 h, quickly (0.25 h) cool to 46°C (optimum temperature), and then maintain at 46°C overnight. The plates were examined for bacterial growth and any well free of growth was designated as a no-growth event. Logistic regression was used to calculate growth probabil-

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ity (Gp) as affected by $NaNO_2$, SE, STPP, and salt. A CCS was defined as the combination of added ingredients with Gp < 0.104 and was validated using inoculated ground beef.

Results: Logistic regression showed that the added ingredients and the interaction of SE with $NaNO_2$, salt, and STPP significantly affected Gp in SFPA, suggesting that proper formulation with the four ingredients may effectively limit the growth of *C. perfringens*. A CCS model was developed based on the logistic regression model. Validation of CCS confirmed no growth of *C. perfringens* in ground beef.

Significance: The results of this study proved that cured meat can be formulated with proper combinations of NaNO2, SE, STPP, and NaCl to prevent the growth of *C. perfringens* under the optimum temperature condition, thus preventing outbreaks caused by growth of this microorganism.

P2-211 Analysis of Heat Resistance-Related Genes and Development of a Heat Resistance Prediction Model of *Campylobacter jejuni* Using Whole Genome Multi Locus Sequencing Typing Data

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Introduction: *Campylobacter jejuni* is one of the broadest-spreading foodborne bacteria. Predicting the thermal resistance of *C. jejuni* has been critical in food production. Bacterial genomic data have recently been used for food safety. The development of new predictive models for heat resistance of unknown strains based on whole genome sequence data is needed.

Purpose: We aimed to reveal the relevant genes for thermotolerance and to develop the predictive model using the machine learning method from WGS data.

Methods: The modified Weibull model described the reduction behaviors of sixteen strains of *C. jejuni* heated at 55°C. Concurrently, the WGS was performed on 14 strains (WGS of two strains were reported). Whole genome sequencing data from 16 strains were used to perform the whole genome multi-locus sequencing typing (wgMLST). All genes of wgMLST were analyzed using the UPGMA method to create phylogenetic trees. For each branch of the phylogenetic trees, Spearman's correlation analysis with the delta parameter of the Weibull model was performed. A Weibull parameter predictive model was developed based on the wgMLST data using the xgBoost, validated by full cross-validation.

Results: The genes with the most significant correlation node were 30S ribosomal protein S7 (rpsG) and invasion antigen CiaC (Cj1242) (R = -0.87). Then, 20 different nodes (16 genes) had a significant correlation (R=-0.86). Heat shock protein (dnaK) and heat-inducible transcription repressor (hrcA) were included among the 16 genes. Other genes included flagellar M-ring protein (fliF) and flagellar motor switch protein (fliG), suggesting a relation between flagellar-related genes and thermotolerance. The predictive model developed had an RMSE of 0.90 log (CFU/CFU) on reduction ratios.

Significance: It was suggested that wgMLST shows the possibility of a predictive model of reduction behavior in *C. jejuni* based on the WGS data.

P2-212 Using a Flexible Supply Chain Risk Model for Leafy Greens to Compare Tradeoffs between Contamination Variability, Finished Product Testing, and Improved Process Controls

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Introduction: The produce industry needs a tool to evaluate most effective food safety management practices to prioritize food safety investments. This tool could guide grower and processor practices and future research.

Purpose: Develop a flexible supply chain risk model (tool) and use it for initial evaluation of practices.

Methods: A user-adaptable model in @RISK for Excel was programmed with five stages representing a leafy green supply chain, a contamination event with *E. coli* O157:H7, and one, 300-gram test per lot at retail as the risk outcome measure. Baseline contamination scenarios (μ=-2.65 log CFU/g) had high (σ=0.8 log CFU/g) and low (σ=0.2 log CFU/g) variability. Probability of contamination was calibrated so the risk of a positive retail test was ~1 in 4,000. We modeled adding two industry-relevant management practices to each baseline: finished product testing (8 375-gram tests/lot) and improved process controls (additional μ=-0.87 log CFU/g reduction). Lots were categorized by the risk of producing a positive retail test.

Results: The overall risk of a positive test in the low-variability scenario (1 in 20,065) was ~5-fold lower than for high-variability scenario (1 in 4,020), implying that rare high-level contamination drives overall risk. Improved process controls reduced overall risk another ~5-fold (to 1 in 113,178 and 1 in 20,063 for low- and high-variability, respectively). Finished product testing reduced overall risk less (~3-fold) in the high variability scenario (to 1 in 11,048) but moved all (23/23) lots categorized as highest-risk for a positive retail test to lower risk categories. Conversely, finished product testing had limited effect (1 in 21,431) in the low variability scenario, likely because there were no highest-risk lots to move.

Significance: We developed a tool that can evaluate tradeoffs between different produce safety management practices, and used it to find, broadly, that reducing relatively rare, high-level contamination events reduces our chosen indicator of risk, a positive test at retail.

P2-213 Quantitative Microbial Risk Assessment for *E. coli* O157:H7 in Formal and Informal Lettuce Production and Supply Chains in South Africa

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Introduction: Consumption of fresh produce is highly recommended. However, leafy greens, such as lettuce have been associated with foodborne disease outbreaks caused by *Escherichia coli* O157:H7. Quantitative microbial risk assessment (QMRA) is used to estimate the adverse human health effects from consumption of foods that may have been contaminated with pathogens.

Purpose: The aim of this study was to assess the risk of illness with *E. coli* O157:H7 from consumption of lettuce from the South African formal and informal lettuce production and supply chains using a QMRA approach.

Methods: A risk model with variables including preharvest contamination, processing, transportation, retail, home storage, and consumption data was developed. A Monte Carlo simulation of the developed model was performed using the Latin Hypercube sampling technique with 100,000 iterations in Lumivero @Risk. The probability of illnesses per serving and number of illnesses per year were estimated based on the available data and information and sensitivity analyses were performed.

Results: The median probability of illness per serving was $1.55x10^{-10}$ for the formal supply chain and $1.87x10^{-10}$ for the informal supply chain. The estimated mean number of illnesses were 392 and 427 for the formal and informal supply chain respectively. Sensitivity analyses for both supply chains indicated that an increase in the retail temperature and concentration of *E. coli* O157:H7 in the soil will result in an increase of the estimated probability of illness and as a result, an increase in the number of illnesses. This analysis also indicates that home storage temperature is an important factor in reducing the probability and number of illnesses in both supply chains.

Significance: This risk model indicates the resulting public health impact from the consumption of lettuce in South Africa. It also provides potential intervention strategies for risk managers to improve food safety and decrease the risk of *E. coli* O157:H7 illness to the consumer.

P2-214 Estimation of Total Pre-Pandemic Poultry Consumption Subtotals for Parts, Ground, and Comminuted Products Using NHANES Datasets – A Comparison of One- and Two-Day Dataset Distribution Estimates

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Introduction: The National Health and Nutrition Examination Survey (NHANES) suspended field operations in March 2020 due to the Covid-19 virus pandemic. As a result, a modified 2017-2020 data set, with two days of intake data for each participant, was made available for nationally representative pre-pandemic analysis.

Purpose: Compare non-adjusted day 1 distribution results with combined day 2 distribution adjusted results, taking into consideration the modified NHANES dataset.

Methods: The statistical software SAS survey means procedure was used to produce distribution estimates for the day 1 data set of 12,634 participants for the total annual chicken and turkey consumption and consumption for parts, ground, and comminuted products. The Iowa State University method was used to compensate for random and systematic reporting errors and distribution skewness in the combined day 2 data set of 10,830 participants.

Results: Day 1 NHANES data was sufficient for the Quantitative Microbial Risk Assessments (QMRAs) requiring only mean estimates for total and subtotal pre-pandemic annual poultry consumption. The means for each of the total and subtotals for parts, ground, and comminuted products were identical for both the day 1 and combined day 2 data sets for both chickens and turkeys. The combined days data set provided the best standard error distribution estimates with decreased total distribution spread at the 5th and 95th percentiles and distribution skew reduction. However, the subtotal distribution estimates, although improved for chickens, were marginal and problematic for turkey subtotals because of diminished sample size for consumers.

Significance: This novel analysis allowed FSIS to more accurately characterize poultry consumption, supporting its efforts to produce robust risk assessments that accurately reflect changing poultry consumption patterns.

P2-215 Growth of Thermotolerant and Mesophilic *Bacillus cereus* in Liquid Egg Yolk during Treatment with Phospholipase A2

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Introduction: Phospholipase A2 (PLA2) is used to treat liquid egg yolk (LEY) during commercial production to improve its emulsification capacity and thermal stability. The conditions used in PLA2 treatment may allow *B. cereus* to grow, potentially presenting a food safety risk.

Purpose: This study was conducted to determine the growth kinetics of thermotolerant and mesophilic *B. cereus* in LEY during PLA_2 treatment.

Methods: *B. cytotoxicus* NVH 391-398, the most thermotolerant of *B. cereus sensu lato*, and an 8-strain cocktail of mesophilic *B. cereus* were inoculated to LEY with PLA_2 and exposed to temperature conditions suitable for each group. For *B. cytotoxicus,* inoculated samples were subjected to dynamically changing temperatures between 20 and 53°C to observe the bacterial growth. For mesophilic *B. cereus,* the samples were exposed to isothermal conditions between 9 and 50°C. The observed growth curves for each group were analyzed using one-step analysis to develop growth models. Numerical analysis and optimization were performed for the dynamic growth curves, while the isothermal growth curves were analyzed with the USDA IPMP-Global Fit, to estimate the kinetic parameters in the Baranyi primary model with a suitable secondary model.

Results: For *B. cytotoxicus* NVH 391-398, the growth showed a significant lag phase between the minimum temperature (18.6°C) and maximum temperature (52.1°C) and occurred optimally at 47.8°C. It grew prolifically at 50°C, with its growth kinetics resembling *Clostridium perfringens,* one of the most rapid-growing foodborne pathogens. For mesophilic *B. cereus*, the growth showed no lag phase, with the minimum, optimum, and maximum temperatures found at 9.3, 42.7, and 48.4°C, respectively. Thermal inactivation occurred at 55°C, a temperature suitable for PLA_2 activity.

Significance: This study determined the growth kinetics of both thermotolerant and mesophilic *B. cereus* in LEY that can be used to design conditions preventing their growth during PLA2 treatment.

P2-216 Modelling Thermal Inactivation of *Salmonella* Montevideo in Red Chili Pepper as Impacted by Temperature and Water Activity

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❖ ***Developing Scientist Entrant***

Introduction: The rising number of outbreaks and recalls linked to *Salmonella* contamination of low-moisture foods, including spices, has raised concerns about their safety. The World Health Organization has determined that red pepper poses the highest risk of *Salmonella* contamination. However, limited data is available on thermal inactivation kinetics of *Salmonella* in red chili peppers.

***Purpose*:** The objective of this study was to investigate the effect of water activity (a_w) on the thermal inactivation of *Salmonella* Montevideo in red chili pepper at three temperatures.

Methods: Red chili peppers were inoculated with *S.* Montevideo and equilibrated in a humidity-controlled conditioning chamber to achieve variable a_w values (~0.30, 0.50, and 0.90). For each treatment, samples (n=12) were placed in 1-mm-thick test cells, heated in a water bath (60, 65, or 70°C), and taken in duplicate at 6 heating times. Survivors were assessed by observing growth on a non-selective differential medium (37°C, 24 h). The D- and *z*-values were estimated using the primary log-linear model combined with the Bigelow secondary model to estimate inactivation. During modeling, the incorporation of isothermal and nonisothermal time-temperature profiles was compared using the root mean squared errors (RMSE).

Results: *S.* Montevideo survival in red chili pepper decreased with increasing aw and treatment temperature throughout time (p <0.05). The D70°C-values for *S.* Montevideo in chili pepper were 16.5 (aw 0.3), 4.1 (aw 0.5), and 0.14 min (aw 0.9). The *z*-values were 15.2, 16.0, and 11.7°C for aw 0.3, 0.5, and 0.9, respectively. Modelling using nonisothermal temperature profiles predicted *S.* Montevideo lethality more accurately than its isothermal counterpart, e.g., at aw 0.3 aw, the RMSE was ~0.5 and ~0.7 CFU/g for isothermal and nonisothermal profiles, respectively.

Significance: This study is a first step toward predicting *Salmonella* reduction in spices during commercial processing, where the temperature and aw are changing over time.

P2-217 Modeling Contamination of Peaches from Food Contact Surfaces during Simulated Dry Post-Harvest Handling

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Introduction: Factors that influence the transfer of foodborne pathogens from dry food contact surfaces to produce are not well characterized.

Purpose: The study aimed to develop a model for assessing factors that contribute to microbial contamination of peaches during a simulated dry handling scenario using Monte Carlo simulation (@RISK, Palisade).

Methods: Log-normal distributions of laboratory-derived transfer rates of *Enterococcus faecium* NRRL B-2354 (EF) were used to evaluate the impact of contamination area and presence of dry peach juice and wax residues on the probability and level of contamination of peaches. Model assumptions included a polyurethane conveyor belt of 1.5 m^2, moving at 6 m/min with 1,500 peaches/min in a single layer across the belt. The inputs included contam-

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ination level (~8 log CFU/cm^2) and area (1 cm^2 baseline [0.0022%]; ranging from ~0 to 150 cm^2 [~0.0001% to 0.3334%]), and transfer rates (0.9% ± 1.4%; baseline to 1.3% or 17.5% in the presence of dry juice or wax, respectively). Simulation iteration was set at 10,000 production days, with 720,000 peaches/day.

Results: The baseline model predicted 820 ± 682 peaches/day (0.114 ± 0.095% [90% confidence interval, CI, 0.008–0.305%]) with mean contamination levels at 4.1 ± 0.4 log CFU/peach (90% CI, 3.5–4.7 log CFU/peach). The predicted numbers of contaminated peaches ranged from <1 to 4,144/day when the contaminated area changed from 0.0001% to 0.3334%, respectively. In the presence of peach juice or wax, the predicted number of contaminated peaches/day was significantly ($p < 0.05$) reduced, but contamination levels significantly increased to 5.2 ± 0.7 log CFU/peach (90% CI, 4.1–6.3 log CFU/peach) or 6.5 ± 0.6 log CFU/peach (90% CI, 5.6–7.4 log CFU/peach), respectively.

Significance: The simulation result can be used to guide further research in developing and comparing effective cleaning and sanitation strategies for dry food contact surfaces.

P2-218 Evaluation of Thermal Inactivation Kinetics of *Escherichia coli* O157:H7, Uropathogenic *E. coli* (UPEC) and *Salmonella* spp. in Ground Meats by One-Step Dynamic Analysis

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Introduction: Thermal inactivation is one of the most effective and practical methods for eliminating foodborne pathogens in foods.

Purpose: This study aimed to evaluate and determine the thermal inactivation parameters of pathogens in ground meats using the one-step dynamic heating to minimize the residual errors globally.

Methods: A multi-strain cocktail of *Escherichia coli* O157:H7, uropathogenic *E. coli* (UPEC), and *Salmonella* spp. were individually inoculated to irradiated ground meats. The samples, pressed to form a very thin layer, were heated to increase in temperature linearly from 30 to 70 ℃ in a programmable water bath at 1.5 ℃ per min. Samples taken when the water bath temperature reached 54, 57, 60, 63, and 67 ℃ were evaluated for the survival counts, which were then used to determine the thermal resistance of each microorganism through non-linear regression, following the first-order inactivation kinetics. Each experiment was repeated three times randomly.

Results: One-step dynamic analysis was used to analyze the dynamic survival curves. The z values obtained were 6.45, 5.40 and 5.82 ℃ for *E. coli* O157:H7 (in ground beef), UPEC (in ground beef), and *Salmonella* spp. (in ground chicken), respectively. The estimated log(D_0), representing thermal resistance at 0°C, was 11.4±0.4, 13.6±0.8, and 12.5±0.5 min for *E. coli* O157:H7, UPEC, and *Salmonella* spp., respectively, indicating the D value at 0ºC is almost infinite. The D value (at temperature T) can be calculated using equation: $\log(D) = \log(D_0) - T/z$. The results show that the D and z values obtained from this study are very similar to the values reported in the literature.

Significance: The results of this study show that one-step dynamic analysis is an effective method to determine the thermal resistance of foodborne pathogens accurately and quickly in foods, while minimizing the residual errors globally.

P2-219 Predictive Modelling of the Psychrotolerant *Bacillus cereus* Group in Fried Rice and Identification of Strain Variability Using Whole Genome Sequencing

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Introduction: Fried rice (FR) is one of the most popular convenience foods, with growing demand, and distributed in chilled or frozen form. However, there are concerns about the presence of the psychrotolerant *B. cereus* group (pBCG), a foodborne pathogen that can grow at low temperatures.

Purpose: The aim of this study was to develop a predictive model for the growth of pBCG and mesophilic BCG (mBCG) on vacuum-packed FR and investigate the genetic characteristics of pBCG.

Methods: To screen for pBCG strains among 76 BCG strains isolated from food, visible colonies were observed after streaking on tryptic soy agar and incubation at 5, 7, and 10 °C for up to 20 days. Primary and secondary models of the selected pBCG and mBCG (ATCC 10987, 21772) on FR were developed using the Baranyi and square root models. The developed models were validated under fluctuating temperatures. Whole genome sequencing was performed to investigate the genetic characteristics of pBCG, using PacBio Sequel and Illumina Novaseq.

Results: The pBCG model was developed in the range of 5 to 37 °C, while the mBCG model was developed from 13 to 37°C, as the mBCG did not grow below 13°C in FR. The developed primary and secondary models fit well ($R^2 \geq 0.98$). Furthermore, the dynamic models were validated, with corresponding RMSE values ≤ 0.5 log CFU/g and acceptable simulation zone values ≥ 80%. The BCG9 strain that showed strong growth at low temperatures, was identified as *B. weihenstephanensis*, and had signature sequences in both 16S rRNA and *cspA* associated with psychrotolerance. In addition, all food isolates showed the presence of the *nhe* and *hbl* toxin gene complexes.

Significance: The developed model can successfully predict BCG growth in FR at different temperatures, including refrigerated conditions, and the presence of pBCG can be a food safety concern.

P2-220 Predictive Model for Growth of *Bacillus cereus* at Temperatures Applicable to Cooling of Cooked Foods

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Introduction: *Bacillus cereus* is a causative agent of diarrheal as well as emetic foodborne illness syndromes. The main contributing factor leading to *Bacillus* food poisoning is its growth from spores which may occur if the rate and extent of cooling of the cooked foods is not adequate or when cooked foods are not appropriately refrigerated during storage.

Purpose: This study aimed to quantify *B. cereus* growth in Tryptic Soy Broth (TSB) at isothermal conditions (15 to 50 °C).

Methods: A cocktail of four strains of *B. cereus* was grown at isothermal temperatures from 10 to 45 ℃ (10, 15, 20, 25, 30, 35, 40, 45 ℃). The growth models of Baranyi and Roberts (1994), Modified Gompertz, and Huang were fitted to the isothermal growth data for each of the two replicates. Microbial numbers (CFU/g) were transformed to natural log values prior to model fitting. The Cardinal, Huang, and Ratkowsky square root models were fitted to the maximum specific growth rate (μmax) values.

Results: Both primary and secondary models fitted the growth data well, as depicted by the goodness of fit measures (high R2, low RMSE/SSE). Values of growth rates ranged from 0.33 to 3.16 ln CFU/h, and lag phase durations ranged from 1.59 to 33.99 h in the temperature range of 15 to 50 °C. For the three secondary models fitted to the growth rates, the accuracy factor and bias factor values were close to 1, the ideal value, making them suitable fits to describe the variation of growth rate.

Significance: A dynamic predictive microbial model will assist regulatory agencies and food industries in predicting potential *B. cereus* behavior in food products stored at improper temperatures and in determining compliance of food processing operations with regulatory performance standards.

P2-221 A Decision-Support Tool for Food Safety Technology Investments

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Introduction: Private producer investment in food safety technology can be a difficult business decision, given that such investments rarely result in direct economic benefits.

Purpose: The objective of this study was to develop a proof-of-concept framework quantifying processor economic benefits associated with risk reduction due to investment in food safety technologies.

Methods: A decision model was built around a quantitative microbial risk assessment with endpoints of product recalls due to outbreaks or testing results, for a case-study scenario of shiga-toxin producing *Escherichia coli* in wheat flour, given several possible food safety interventions (tempering, heat treatment, and sanitization). Recall costs, such as lost product, sales, and production, sustained for small, medium, and large firms were estimated with assistance from industry partners. These inputs were incorporated into a decision analysis program coded in R, and a Monte Carlo analysis was conducted with 10,000 iterations to yield the range of firm-specific financial implications associated with each combination of food safety decisions.

Results: This work resulted in a novel, risk-based decision-making tool for food safety technology investments by food processors. The tool is comprised of 2 main branches, for decisions to implement a food safety technology or not, with 7 chance nodes per branch, representing the probabilities of contamination, detecting contamination, illnesses linked to outbreaks, and outbreaks being traced to the producer. Given user inputs such as production rates, specific food safety technology costs and efficacies, and testing schemes, the tool's output is the cost, in dollars, associated with each decision. This allows for the calculation of the net economic value for a given food safety technology investment, incorporating the valuation of the resulting risk reduction.

Significance: This tool can support food processors and technology suppliers, providing for the first time a quantitative, risk-based economic justification for food safety technology investment decisions.

P2-222 Artificial Intelligence (AI) as a Tool for Hazard Assessment in the Food Industries: Threats and Opportunities

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Introduction: The use of Artificial Intelligence (AI) in the food industry for hazard assessment brings a mix of benefits and risks. This review explores AI's impact in this sector, focusing on its role in enhancing food safety and operational efficiency, alongside the potential challenges it presents.

Purpose: The aim is to assess how AI can be employed in the food industry for hazard assessment and to comprehend its broader implications, particularly in enhancing safety and identifying potential risks associated with its use.

Methods: The analysis includes a review of literature and case studies that discuss AI applications in the food industry. It examines AI's capacity for analyzing complex, unstructured data, such as safety observations and risk assessments, and its contributions to improving food safety practices. Additionally, the review considers the threats posed by AI, including cybersecurity risks, environmental concerns, and socioeconomic implications.

Results: AI proves effective in extracting insights from complex datasets, aiding in hazard identification and risk mitigation. Its role in enhancing food safety, particularly in retail, is significant, with various applications demonstrated. However, risks are notable, as exemplified by the cyberattack on JBS, the world's largest meat processor. This incident highlights the vulnerability of AI-integrated systems to cyberattacks, disrupting operations and causing significant impact. Environmental risks, such as the potential overuse of fertilizers and pesticides, and socioeconomic challenges, including the perpetuation of inequalities in farming practices, are also concerns.

Significance: The benefits of AI in improving efficiency and safety in the food industry are substantial, yet its deployment necessitates responsible management. Addressing cybersecurity threats, as illustrated by the JBS incident, considering environmental impacts, and tackling socioeconomic disparities are critical for sustainable AI implementation. Strategic and cautious application of AI is vital to leverage its advantages while mitigating associated risks.

P2-223 Cool Insights: Unveiling Key Consumer Messages through Refrigerator Temperature Studies and QMRA Analysis

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Introduction: Inadequate domestic refrigeration is one of the most common causes of foodborne illness, and domestic consumer practices can vary significantly.

Purpose*:* Determining the most important factors affecting the number of listeriosis cases from ready-to-eat (RTE) cooked meat products.

Methods*:* A survey was conducted among Dutch consumers (n=1,020) to assess the knowledge, consumer practices and temperatures of domestic refrigerators, Quantitative Microbiological Risk Assessment (QMRA) was performed to identify the primary factors contributing to the risk of listeriosis, from which key consumer messages were derived.

Results*:* The average temperature of domestic refrigerators was 5.7 °C (SD 2.2 °C) with a maximum of 17 °C. Temperature measured on the upper shelf was significantly higher (mean 7.7 °C) than the temperature measured on the bottom shelf (5.7 °C). Elderly people (65 years and older) had refrigerators with temperatures that were on average 0.6 °C higher than those of younger people (35 years or younger). The QMRA model predicted an average number of 191 cases of listeriosis per year (SD 54) for the total population. As expected, the high-risk populations displayed a significant higher risk of listeriosis compared to the low-risk population (175 cases versus 16 cases). Scenario analysis revealed that storing opened RTE cooked meat products at home for either less than 7 days and at temperatures below 7 °C resulted in a significant reduction of over 95% in predicted illness cases.

Significance: Targeted communication, particularly directed towards the elderly, on the importance of storing RTE cooked meat products at the recommended temperature on the bottom or middle shelf as well as consuming within two to three days after opening, has the potential to significantly decrease the number of listeriosis cases.

P2-224 Modeling the Combination Effects of Salt, pH and Time on the Growth of *Bacillus cereus* and *Clostridium perfringens* in Sauces at 75°F and 90 °F

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Introduction: Limited research has been conducted on the safety of chilling and holding high moisture slurries at 70 °F and above following a lethality step. Not all food manufacturers fully chill the sauce products to 40 °F or below due to process constraints and product handling.

Purpose: Determination of hold times for cooked sauces inoculated with B. *cereus* and C. *perfringens* spores to provide safety guidance on time and temperature control.

Methods: The sauce model contained salt, sugar, homogenized milk emulsion, chicken broth, starch, and water. The response surface design with nine treatments and three replicates were utilized to investigate the combination effects of salt (0.5%, 0.88%, 1.25%, 1.62%, 2%), pH (5.5, 5.85, 6.2, 6.55, 6.9), and time (up to 24 hrs.) on B. *cereus* and C. *perfringens* growth at 75°F and 90 °F. All sauces were cooked to 165 °F and inoculated with B. *cereus* or C. *perfringens* spores at 2-3 log CFU/ml. After inoculation, each treatment was divided into two batches, one chilled to 75 °F or below in one hour, the other to 90 °F or below in one hour. Sauces were then held at 75°F and 90 °F for microbial evaluation.

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Results: For both temperatures, 652 data points were split into a training set and a testing set. A quadratic response surface model fit the training data to estimate the log count at a given combination of salt, pH, and time. Models for both temperatures were determined to fit well with R^2 0.63 – 0.85 on the testing set. Interactions among salt, pH and time were seen on both strains and temperatures.

Significance: The resulting models will be utilized to estimate the log counts for user input scenarios of salt, pH, and time. It will allow for timely decision-making to ensure food safety isn't compromised during processing.

P2-225 Spatial Modeling of the Poultry Chilling Process: Impact of Water Recirculation and Counterflow on *E. coli* and *Campylobacter* Dynamics

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Introduction: Current models of pathogen dynamics during poultry chilling assume homogeneous mixing profiles for water in the chiller tank. To realistically predict changes in pathogen levels, models cannot ignore spatially dependent features such as water recirculation and counter-flow dynamics.

Purpose: Develop a novel mathematical model of the chilling process which captures the impact of water dynamics on pathogen inactivation and cross-contamination.

Methods: Partial differential equation model, tracking water chemistry and pathogen levels on carcasses and in water as a function of space in the chiller tank was developed. Equilibrium solution analysis was conducted via dynamical system theory. Model parameters were fit against commercial chiller data in the literature for free chlorine control of both *E. coli* and *Campylobacter* contamination on carcasses. Model parameters/outputs and sensitivity analysis were analyzed using MATLAB.

Results: Our model has a unique, globally attracting equilibrium, justifying practical use of the steady-state solution. Distributions for parameters involving water chemistry and *E. coli* and *Campylobacter* transfer and inactivation rates were determined from literature data. Following commercial procedures, post-chill *E. coli* distributions on carcasses were computed as a function of pre-chill levels and compared to USDA guidelines. Post-chill *Campylobacter* distributions on carcasses were computed in terms of pre-chill levels and compared with FSIS limit of detection (1 CFU/ml rinsate). On average, results indicate approximately a 1.5 log reduction of *E. coli* and a 2 log reduction of *Campylobacter* on carcasses when varying pre-chill levels from 3 to 7 log10 CFU/carcass. Sensitivity analysis illustrated two tank regimes: the carcass entry portion, where bacterial levels are controlled by shedding/attachment rates and the carcass exit part of the tank characterized by free chlorine inactivation kinetics.

Significance: Our model results provide key information for decision making aimed at pathogen control/compliance during poultry chilling as well as developing more accurate risk assessment strategies to improve poultry safety.

P2-226 Development of Dynamic Models to Describe the Kinetic Behavior of Aerobic Bacteria in Beef

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Introduction: Various microorganisms, especially aerobic bacteria, affect the beef quality. Thus, control of total aerobic bacterial counts is important in beef.

Purpose: The objective of this study was to develop dynamic models to describe the kinetic behavior of aerobic bacteria in beef under changing temperatures.

Methods: A mixture of aerobic bacteria isolated from 12 beef samples was inoculated in 10 g of beef at 4-5 log CFU/g. The inoculated samples were aerobic, or vacuum packaged and stored at 5°C, 15°C, 20°C, and 30°C up to 504 h, depending on the storage temperature. The total aerobic bacterial (TAB) counts were enumerated on plate count agar (PCA). The Baranyi model was fitted to the TAB counts to calculate lag phase duration (*LPD*; h) and maximum specific growth rate (μ_{max}; Log CFU/g/h). These kinetic parameters were analyzed with the square root model as a function of temperature. To evaluate the model performance, the models were validated with observed data, and root mean square error (*RMSE*), B factor (B_f), and A factor (A_f) were calculated. Dynamic models were developed in accordance with the developed primary and secondary models.

Results: As the storage time increased, *LPD* generally decreased, and μ_{max} increased. The secondary models were also developed with R^2 of 0.398-0.457 for *LPD* ($LPD^{0.5}$=4.424-0.159×temperature and $LPD^{0.5}$=4.468-0.133×temperature for aerobic packages and vacuum packages, respectively) and R^2 of 0.890-0.970 for μ_{max} ($\mu_{max}^{0.5}$=0.103+0.018×temperature and $\mu_{max}^{0.5}$=0.132+0.017×temperature for aerobic packages and vacuum packages, respectively). *RMSE*, B_f, and A_f were 0.438-0.596, 0.996-1.045, and 1.055-1.079, respectively, indicating that the developed models were appropriate to describe the kinetic behavior of aerobic bacteria. The model predictions under changing temperature were appropriate with 1.155 and 1.229 of B_f for aerobic packages and vacuum packages, respectively.

Significance: This result indicates that the developed models should be useful in describing the kinetic behavior of aerobic bacteria in beef at changing temperature.

P2-227 Production of Preservatives in Fermented Dairy Drinks

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Introduction: Preservatives are food additives to prevent microbial spoilage. However, the preservatives may be naturally present in food ingredients or produced during fermentation.

Purpose: This study investigated the production of preservatives in a fermented dairy drink during the production.

Methods: Skimmed milk powder was diluted in purified water at 10% concentration, and the diluent was heated at 105°C for 10 min. The inoculum strain (lactic acid bacteria) isolated from a commercial product was inoculated into the skimmed milk solution. This mixture was fermented at 37°C for 24 h. The fermented skimmed milk and the sugar mixture (10% fructose + 0.00015% aspartame + 0.24% fruit concentrate + 0.0002% fruit flavor) were mixed at a ratio of 1:9 and pasteurized at 65°C for 30 min. Preservative content was measured for skimmed milk, heated skimmed milk solution, fermented sample, the mixture of the fermented skimmed milk and sugar mixture, and pasteurized sample during the manufacturing. Propionic acid was determined by gas chromatography with a flame ionization detector, and benzoic acid and sorbic acid were determined by high performance liquid chromatography with a photodiode array detector.

Results: The fermented dairy drink was prepared properly with 3.71±0.43 of pH and 8.0±1.0 Log CFU/mL of the total lactic acid bacteria. During the fermented dairy drink manufacturing, 32.2±0.6 ppm of benzoic acid was detected in fermented samples only but not in samples at the final stage, possibly due to dilution effect while mixing the fermented samples with sugar mixture. Propionic and sorbic acids were not detected in any samples.

Significance: This result indicates that benzoic acid might be produced during the skimmed milk fermentation, but it may not last in a further process. In addition, the result may depend on lactic acid bacteria, and thus, an additional study is necessary.

P2-228 *Escherichia coli* and *Citrobacter koseri* Harboring *clb*A, *clb*P, and *clb*Q Genes Cause DNA Cross-Linking *in vitro*

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❖ ***Developing Scientist Entrant***

Introduction: Colibactins are genotoxic hybrid polyketide-nonribosomal peptides produced by Enterobacteriaceae harboring *clb* genes (*clb*A, *clb*P, or *clb*Q). The colibactins were produced by human commensals, and they may damage DNA.
Purpose: This study investigated if *Escherichia coli* and *Citrobacter koseri* harboring *clb* genes cause the DNA interstrand cross-linking *in vitro*.
Methods: *Escherichia coli* KCTC1682 and *Citrobacter koseri* ATCCBAA-895 harboring *clb* genes (*clb*A, *clb*P, and *clb*Q) cultures were prepared at OD_{600}=0.01. pUC19 was linearized by EcoRI, and the linearized pUC19 was co-cultured with the bacteria at 37°C for 5 h. *Escherichia coli* KCTC2571, having no *clb* genes, was co-cultured with the linearized pUC19 for negative control. After the co-culture, DNA was extracted from the co-culture and purified. The purified DNA was added to 5 µL of 0.4% (v/v) sodium hydroxide and left on ice for 10 min to denature the purified DNA. The purified DNA (native DNA) samples before denaturation and treated DNA for the denaturation were electrophoresed to compare DNA size to determine crosslinking in the DNA.
Results: The native linearized DNA strands exposed to either the *clb* gene harboring bacteria or negative bacteria showed the double-stranded DNA. In the denaturation condition, linearized DNA strands exposed to *E. coli* KCTC1682 and *C. koseri* ATCCBAA-895 were confirmed to have the same size of bands as the native condition, meaning that DNA was not denatured. The DNA strands exposed to *E. coli* KCTC2571 were confirmed to have bands with smaller sizes compared to the native DNA as they were denatured to single strands.
Significance: The result suggested that DNA was not denatured completely if it had cross-linking, and thus, *E. coli* KCTC1682 and *C. koseri* ATCCBAA-895 harboring *clb* genes did not denatured DNA because they caused DNA cross-linking *in vitro*.

P2-229 Inferential Modeling of Coronavirus Persistence and Surface-Mediated Transfer to Human Skin

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Introduction: Despite extensive research on airborne transmission of the COVID-19-causing virus, the risk associated with the surface-mediated transmission of coronaviruses in the food supply chain has remained underexplored.
Purpose: To employ inferential modeling of previously collected data (IAFP 2023) to assess the persistence of coronavirus on various contact surfaces and its secondary transmission to humans.
Methods: Laminated menu (LM), clamshell containers (CS), coffee cup lids (CCL), and cardboard (CB) coupons (1.5×1.5 cm) were inoculated with 8 $\log_{10}$ PFU of bacteriophage Phi6, a coronavirus surrogate. Coupons were stored for 144h at 4ºC and 25ºC under 45% and 65% relative humidity (RH) levels and were tested with infectivity (plaque assay) over storage times. The transfer rate of infectious virus particles was determined through exposure of the inoculated coupons to artificial human skin. Kinetics of virus inactivation were evaluated using linear and non-linear models. The best-fitting models were selected using second-order information criteria, followed by inferential statistical analyses of bootstrapped data.
Results: Inactivation kinetics were best fitted with linear, logistic, and Weibull models depending on temperature-RH combinations. The time for a 1-$\log_{10}$ PFU reduction (t_{1D}) varied across the treatments, with the lowest and highest values observed at 25ºC (0.4h to 104h) and 4ºC (121h to >144h), respectively. The RH levels had a marginal impact on virus inactivation for CS; however, higher RH yielded increased virus inactivation for CB and CCL (p<0.01). No virus transfer was observed from CB to skin. Mean transfer rates from other surface types were 1.1%-1.5%, showing no difference among the surfaces (Wald chi-squared = 1.22, p = 0.54).
Significance: This research contributes to developing quantitative risk assessment models, enhancing our understanding of coronavirus transmission risks through food contact surfaces. The findings provide insights for strengthening public health protection measures against pathogenic viruses in the food sector.

P2-230 Online Platform for Curating Food Safety Datasets to Facilitate Model Development

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Introduction: Publicly available datasets can be valuable for the development of Artificial Intelligence (AI) tools and algorithms, but appropriate databases are missing for food safety.
Purpose: The goal of this project is to develop an online platform to collect and curate datasets to facilitate development of AI tools that can help address various food safety issues.
Method: A dataset was acquired from a previous study, which includes 1,004 soil samples collected in the U.S. paired with comprehensive metadata (i.e., location, soil properties, climate conditions, surrounding land use) as well as experimentally determined *Listeria* spp. presence data. After removing samples with missing soil property data and impute for samples with missing climate data with values of nearest location, the sample size was reduced to 622 samples. The cleaned dataset was hosted on a GitHub organization page named FoodDatasets that will serve as a central hub for future integration of more datasets. To exemplify the use of the platform and dataset, several supervised machine learning algorithms (i.e., neural network, logistic regression, support vector machine, k-nearest neighbors, gradient boost, decision tree) were used to train 80% samples and evaluated on the remaining 20% samples with the task of predicting *Listeria* presence in the soil sample. The code for developing these models has been made available on GitHub with clear instructions to clone and modify.
Results: All models had acceptable performance on the test dataset with accuracy ranging from 0.695 to 0.827. The gradient boost yielded the highest accuracy of 0.827 with a sensitivity of 0.813, specificity of 0.869 and F1 score of 0.840, showing promising potential for *Listeria* detection in the field.
Significance: This platform will be a starting point for inclusion of more datasets that can be used as benchmark for driving AI innovations in food safety as well as evaluating algorithms performance.

P2-231 FSIS' Bioinformatics Supplemental Materials Workbook: A Tool to Enhance Transparency in Regulatory Rulemaking

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Introduction: The Food Safety and Inspection Service (FSIS) conducted two probabilistic quantitative microbial risk assessments (QMRAs) in support of new rulemaking to reduce *Salmonella* illnesses from poultry products. Incorporating whole genome sequencing analyses into these risk assessments were pivotal in developing robust final product standards based on serotypes of public health concern. Given the complexity of this work, and the need for transparency per the United States Information Quality Act, FSIS developed a supplemental workbook to ensure its approach could be understood and replicated.
Purpose: Describe a genomics-based approach—and FSIS' utilization of that approach—to cluster *Salmonella* serotypes according to virulence-associated gene markers and assess associated risk.
Methods: FSIS collaborated with EpiX Analytics to incorporate a genomics-based component to determine groupings of *Salmonella* serotypes based on

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an array of virulence factors (i.e., seroclusters). To ensure transparency and accessibility, as well as to enhance its utility, FSIS detailed the approach (including limitations and future extensions) and assessed the resulting seroclusters for appropriate incorporation into the poultry QMRAs.

Results: Results will be shared when the proposed Salmonella Framework Rule is published, per Office of Management and Budget rules.

Significance: This tool, and the machine learning and advanced bioinformatics applications described in the tool, can serve as potential pipelines for exploring genomic data and patterns associated with *Salmonella* virulence as the complex pathogenic mechanisms are not yet completely understood.

P2-232 Fungal Communities and Metabolites during Activation of Ginger Root Microbiota and Derived Ginger Beer

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Introduction: Ginger beer is beverage produced using ginger (Zingiber officinale Roscoe) autochthonous microbial communities activated in a pre-fermentation step. Little is known about the fungal diversity and metabolites during the ginger activation and in the final product.

Purpose: To assess the fungal communities and metabolites during ginger mycobiota activation and in the derived ginger beer.

Methods: Grated organic ginger (8%w/v) was added of organic crystal sugar (8%w/v) and sterile mineral water (300 mL) and kept at 25ºC for 96h in aerobiosis). Ginger beer was prepared using ginger (4%w/v), organic crystal sugar (15% w/v), lemon juice (1% v/v), sterile mineral water (1L) and the pre-fermented ginger (25%v/v). The beverage was bottled and maintained for 14 days at 25◦C. Aliquots of ginger activation (time: zero, 48 h and 96h) and ginger beer (time: zero and 7 days of fermentation) were collected for the analysis. Fungal DNA was extracted using a MoBio Power Food DNA Isolation Kit. The ITS rRNA regions were sequenced on the MiSeq Sequencing System using the primer pair ITS1F-ITS2R. Taxonomy assignment was done using UNITE v9 software. Metabolites were evaluated by high-performance liquid chromatography. Statistical analyzes was done using XLSTAT 2020.1.3 ($p<0.05$).

Results: The main predominant fungal families during microbial ginger activation were Cladosporiaceae, Ceratobasidiaceae at zero time, and Ceratobasidiaceae, Plectosphaerellaceae, Podosporaceae, Mortierellaceae, Fungi_fam_Incertae_sedis, Herpotrichiellaceae and Nectriaceae at 48 h and 96 h. In the ginger beer a high prevalence of Saccharomycodaceae and Pichiaceae was observed after 7 days. Glucose and fructose increased at 48 and 96 h of activation. In the ginger beer, sugars, citric and acetic acids decreased, while malic, succinic, lactic, and formic acids increased.

Significance: Results characterize the mycobiota related to a fermented beverage consumed worldwide. Potentially toxigenic fungal groups were not identified during ginger roots activation and ginger beer derived.

P2-233 Evaluating the Temperature and Relative Humidity Effects on the Survival Rate of *Salmonella enterica* in Chocolate Filled with Contaminated Cocoa Nibs

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Introduction: Chocolate made with ingredients carrying *Salmonella enterica* has been involved in outbreaks and recalls. Cocoa nibs are widely used in chocolate manufacture. *S. enterica* on nibs can survive thermal processing and storage. Few data are available on the behavior of this pathogen in chocolate products made with contaminated cocoa nibs.

Purpose: This study assessed the survival of *Salmonella* in chocolate made with contaminated cocoa nibs during storage at different temperature and relative humidity (RH) conditions.

Methods: Cocoa nibs were contaminated with *S. enterica* (*S.* Typhimurium, *S.* Enteritidis, *S*. Newport, *S*. Oranienburg, and *S.* Montevideo; ~7 log CFU/g) and incorporated into creamy chocolate. Molded chocolate bars (3 cm × 3 cm × 2.3 cm) were packaged in polypropylene (commercial packaging for chocolate; water vapor transmission rate 0.25 g/mil/100 in 2/24 h at 100°F). Samples were kept in desiccators over saturated salt solutions of nitrate chloride and potassium chloride (65 and 100% RH, respectively) at 7 and 14 °C. Viable cells were enumerated onto *Salmonella-Shigella* agar every three days during 60 days of storage (detection limit 1.5 log CFU/g). Data were fitted to the linear regression model using the R statistical computing language version 4.2.2.

Results: Data showed a good fit to the model with R2 ≥0.9. Temperature influenced the change in the survival rate of *S. enterica* in cocoa nibs, favoring survival at the lower temperature, regardless of RH. A decrease of 0.8 and 1.3 log CFU/g of *S. enterica* in nibs was observed after 60 days of storage at 7 and 14 °C, respectively. Humidity had no significant influence on the survival of *S. enterica*.

Significance: The impact of temperature on the change in survival rate of *S. enterica* in chocolate products made with cocoa nibs should be considered for developing risk management strategies.

P2-234 Impacts of Glow Discharge Cold Plasma Treatment on Microbiota Composition of Fresh Edible Red Mini-Roses (*Rosa chinensis* Jacq.)

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Introduction: Mini-roses (*Rosa chinensis* Jacq.) are raw consumed in salads, salty dishes and desserts. Mini-roses are highly perishable and color impacted by classic antimicrobial treatments. Glow discharge cold plasma technique is proposed as an emerging technology to increase shelf-life and guarantee safety with minimal damage to vegetable quality. No prior studies have evaluated the impacts of cold plasma on microbiota of edible mini-roses.

Purpose: To determine the impacts of glow discharge cold plasma on microbial communities of fresh red mini-roses.

Methods: A PE-50 gas discharge plasma system (Plasma Etch, USA) was used operating with synthetic air at 50 kHz and electrical potential difference of 80 kV. Organic red mini-roses at commercial maturity (21 days) were treated in the processing chamber at 0.3 bar, with an airflow rate of 20 mL/min and at room temperature (~25°C) for 20 min. DNA was extracted using DNA Power Soil Pro isolation kit, 16S rRNA amplicons were sequenced using an Illumina PE 250 platform. High-quality readings were analyzed using QIIME 1.9.1 and statistical analyzes were performed using XLSTAT software 2020.1.3, considering $p < 0.05$. Color parameters (luminosity, redness and yellowness) were evaluated every 24 h over 5 days.

Results: Cold plasma had reduced the bacterial diversity indices and the relative abundance of various bacterial groups including phyla *Firmicutes* (27.35 vs 0.06 %), and *Actinobacteriota* (1.42 vs 0.03%). Genus *Lactobacillus* associated to flowers spoilage also decreased (14.23 vs. 0.00 %). Flowers treated with glow cold plasma had higher relative abundance of *Proteobacteria* (99.90 vs 67.94%), *Erwiniaceae* (99.44 vs 60.78%) and *Rosenbergiella* (80.74 vs 51.90%) compared to non-treated mini-roses. Glow cold plasma did not influence the color parameters.

Significance: Results show the positive influence of glow cold plasma in decontaminating fresh red mini-roses. Findings indicated the absence of foodborne bacterial groups in treated or non-treated mini-roses.

P2-235 Prebiotic Potential of Cassava (*Manihot esculenta*) against *Lacticaseibacillus casei* 1 and *Lactobacillus acidophilus* 5

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Introduction: Cassava (*Manihot esculenta*) is the main source of carbohydrates in some low- and medium income countries. Cassava is a source of oligosaccharides, and dietary fibers. These components may have health benefits, acting as prebiotics. Little is known about the ability of cassava roots to act as prebiotics.

Purpose: To evaluate the prebiotic potential of a Brazilian cassava cultivar against the probiotic strains *Lacticaseibacillus casei* 1 (Lc-1) and *Lactobacillus acidophilus* 5 (La-5).

Methods: Freeze-dried grated cassava was *in vitro* digested (oral, gastric and intestinal phases) to simulate as it reaches the colon. Fermentation with probiotics (Lc-1 and La-5) or *Escherichia coli* (6 log CFU/mL) was performed by adding 0.5 mg/mL of cassava to MRS or M9 broth, respectively. Frutooligossacarídes (FOS) at the same concentration and only MRS or M9 were used as positive and negative controls, respectively. After 48 h at 37°C, viable cells were enumerated onto MRS agar (probiotics) or EMB agar (*E. coli*) agar (detection limit of 1.5 log CFU/g). Prebiotic score was calculated considering the initial counts in negative control divided by the final counts in media with cassava multiplied by 100%. A positive prebiotic score was obtained when probiotics had higher counts in the media with cassava when compared *E. coli.* The probiotic metabolism was evaluated by sugars, organic acids, and phenolic contents measured by high-performance liquid chromatography. Statistics considered $p<0.05$.

Results: Probiotic counts increased up to 2 log CFU/mL in MRS with cassava with a higher proliferation rate than FOS. The prebiotic scores of cassava were positive (La-5: 0.27; Lc-1:0.2), higher, or similar to FOS (0.20). During the fermentation, maltose and glucose decreased while organic acids (mainly acetic and butyric) and units of polymeric phenolics increased.

Significance: Cassava has prebiotic potential comparable to FOS, the current standard prebiotic component. Cassava should be considered an alternative plant-based food with functional properties.

P2-236 Machine Learning Approaches to Predict the Clinical Symptoms of Shiga Toxin-Producing *E. coli* Using Public Genome Data

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Introduction: Machine learning approaches that utilize whole-genome sequences are gaining recognition in the field of food safety because of their exceptional ability to predict complex phenotypes and causes of infections.

Purpose*:* This study aimed to predict the clinical symptoms of Shiga toxin-producing *E. coli* (STEC) by using machine learning approaches that employ whole-genome sequences as feature data.

Methods*:* The raw reads or assemblies of 104 genomes with clinical symptoms were collected from the NCBI database. The samples were categorized into two groups: HUS (n=43) and non-HUS (n=61), which included less severe diseases, such as diarrhea. The analysis involved pan-genome analysis and prediction of virulence, antibiotic resistance, and stress-related genes to generate gene presence/absence feature data. Eight classification models, such as logistic regression and random forest, were evaluated using 10-fold cross-validation and hyperparameter tuning on the training dataset. The optimized model was then applied to the test dataset and assessed using a confusion matrix, AUC, accuracy, precision, recall, and F1 scores.

Results*:* The 109 STEC genomes were classified into seven different serotypes, including O157:H7, irrespective of the clinical symptoms. The pan-genome analysis identified a total of 9,830 gene families. Among these, 1,644 genes exhibited statistically significant associations with the clinical label ($p<0.05$), and 111 genes were identified through the detection of pathogenicity-related genes encompassing five Stx subtypes (1a, 2a, 2c, 2d, 2f). The feature data were generated by the presence/absence of the combined 1,755 genes based on the analyses of pangenome and pathogenicity-related genes. In predicting the test dataset, the logistic regression showed the best performance with the accuracy of 0.905, precision of 0.818, recall of 1.000, F1 score of 0.900, and AUC of 0.972.

Significance: This study indicates that machine learning has the potential to function as an advanced diagnostic tool, effectively using complicated genome data.

P2-237 Understanding the Genetic Regulation of Paenibacillin Biosynthesis through Quorum Sensing Systems

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❖ *Developing Scientist Entrant*

Introduction: The surge in antibiotic resistance in foodborne pathogens poses serious safety risks. Paenibacillin, an antimicrobial peptide from Paenibacillus polymyxa OSY-DF, exhibits strong activity against Gram-positive bacteria like Listeria monocytogenes. Paenibacillin is produced from a biosynthesis gene cluster, containing 11 putative genes, including accessory gene regulator (Agr)-like quorum sensing (QS) system genes.

Purpose: Investigating the roles of Agr and universal autoinducer-2 (AI-2) QS systems as regulators for paenibacillin biosynthesis.

Methods: To determine the impact of AI-2 signals on paenibacillin biosynthesis, *P. polymyxa* OSY-DF and OSY-EC (mutant strain) were incubated with 0-200mM concentrations of D-ribose (AI-2 inhibitor) for 24hrs. Crude extracts (CE) were obtained, and AI-2 activity was quantified via luminescence of *Vibrio harveyi,* a sensor strain. To assess the Agr system's role, *P. polymyxa* OSY-DF was grown in TSB-YE media for 36hrs, and CE and RNA samples were extracted at 0, 3, 6, 9, 12, 24, and 36hrs for RT-qPCR gene expression analysis. RT-PCR analyzed all 11 genes in the paenibacillin biosynthesis gene cluster. Antimicrobial activity was tested with a soft agar overlay using *L. innocua* as a sensitive indicator.

Results: *V. harveyi* treated with CE of OSY-DF and OSY-EC exhibited significantly stronger AI-2 activity (p <0.05) compared to the untreated control. AI-2 signal decreased in *V. harveyi* treated with CE of OSY-DF and OSY-EC grown in D-ribose, without differences in paenibacillin antimicrobial activity. Gene expression studies revealed substantial upregulation of paenibacillin biosynthesis (42- to 4002-fold increase) and *agr* genes (99- to 3061-fold increase), peaking at 24hrs, this pattern aligned with *in-vitro* antimicrobial testing. Statistical analysis indicated a strong positive correlation (r^2=0.89-1.00, p <0.05) between paenibacillin biosynthesis and *agr* gene expression. The *agr* system likely plays a crucial role in paenibacillin biosynthesis regulation, while AI-2-mediated QS may not.

Significance: Understanding genetic regulation facilitates optimization, and controlled production of paenibacillin, providing an alternative antimicrobial for food safety.

P2-238 Modifying the Powersoil Protocol to Improve the Extraction of DNA from Bacterial and Fungal Cells

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❖ ***Developing Scientist Entrant***

Introduction: Obtaining accurate microbiome data for food processing surfaces is dependent on efficient DNA extraction from a diversity of microbial cells present in those samples.

Purpose: Modify the standard protocol of the PowerSoil kit to improve DNA extraction efficiency from various microbial cell types.

Method: Four modifications to the standard protocol of PowerSoil kit were tested: 1) extending bead-beating for 10 minutes, 2) pretreating cultures at 95°C for 10 minutes, 3) pretreating cultures at 65°C for 10 minutes, and 4) pretreating cultures with 10 mg/mL lysozyme and 5mg/mL proteinaseK at 37°C for 10 minutes. These methods were used to extract DNA from spore cultures of *Alicyclobacillus suci*, *Bacillus cereus*, *Exophiala phaeomuriformis*, *Aspergillus fishcerii*, and vegetative cell cultures of *Listeria monocytogenes* and *Escherichia coli*. Extraction yield was determined based on the concentration of DNA in each lysate measured by Qubit and qPCR. Differences in extraction yields between methods were evaluated by paired-end t-test. PCR amplification of the 16S and ITS region of the DNA extracts of mock communities was conducted, products were sequenced, and the relative abundance of the sequences were compared to the known composition. Differences between the observed and theoretical relative abundances were evaluated by student t-test.

Results: Extended bead-beating significantly improved ($p < 0.05$) extraction yields from *A. suci* (94% increase), *B. cereus* (105% increase), and *E. phaeomuriformsis* spores (63% increase). Other modified methods did not improve extraction yields consistently across different cell cultures. The relative abundances of *Bacillus* and *Listeria* in the mock communities were significantly different from the theoretical value ($p < 0.05$) when applying the extended bead-beating method.

Significance: Extending the bead-beating step in the PowerSoil extraction protocol may improve DNA extraction from diverse microbial communities found in food plants. However, additional experimental aspects (e.g., primer bias) must also be addressed for accurate microbiome analysis.

P2-239 Resistome Analysis of Seafood Samples Using Shotgun Metagenomics

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Introduction: Comprehensive profiling of antimicrobial resistance genes (ARGs) in the farm to fork food supply chain is a goal of integrated surveillance programs. The distribution and relative abundances of ARGs in seafood microbiomes are not well understood. High-throughput next generation sequencing technologies offer new approaches for antimicrobial resistance monitoring.

Purpose: The aim of this study was to catalogue and quantify ARGs present in different seafood samples collected by the National Antimicrobial Resistance Monitoring System using shotgun metagenomics approach.

Methods: A total of 122 seafood samples (41 salmon, 35 shrimp and 46 tilapia) were included in the study. Community DNA was extracted and sequenced on a HiSeq2500 sequencer. The presence and abundance of ARGs in the metagenomic dataset were determined using the Short, Better Representative Extract Dataset unique peptide markers generated from the AMRFinderPlus database. We used LDA Effect Size to determine ARGs most likely to explain the difference between sources.

Results: We identified 91 ARGs representing 15 antimicrobial resistance classes. We identified more than 31 β-Lactam resistance genes including *blaOXA*, *blaIMP*, *blaCMY* and *blaFOX* genes. In addition, we detected fluoroquinolone resistance genes namely: *qnrA*, *qnrB*, *qnrD*, *qnrS, qnrVC* and *oqxB* genes. The distribution and relative abundance of ARGs observed varied by sample type. The three most common ARG observed in salmon were *aac(3)-VIIa* (70.7%), *blaLRA-1* (53.7%), *emhC* (46.3%) and *tet(L)* (46.3%) while *aac(3)-VIIa* (68.6%) was the most common ARG in shrimp followed by *emhC* (51.4%) and *blaOXA-548* family (48.6%). Among tilapia samples, *aac(3)-VIIa* (58.7%) and *blaOXA-548* family (58.7%) were the most common ARGs followed by *tet(L)* (56.5%).

Significance: Our study provides valuable insights into the diversity and identity of ARGs present in seafood samples. This work helps characterize the resistome of seafood microbiome beyond what can be determined by culture-based methods alone. This information will help shed light on the potential of metagenomics as an auxiliary method for monitoring resistance in the food supply.

P2-240 Assessing Genetic Evolution, Virulence, and Antimicrobial Resistance of *Listeria monocytogenes* Isolated from Food and Food Processing Environments

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Introduction: A repository of 159 historic *Listeria monocytogenes* isolates (198X-2008) from Cathy Donnelly's *L. monocytogenes* isolate collection was sequenced and characterized *in silico* to observe differences in virulence and stress tolerance genotypes.

Purpose: To identify isolates of interest for phenotypic characterization, and to observe differences in genotype between isolates from various source types and decades.

Methods: *Listeria monocytogenes* historic isolates from food products (n=128) and food processing facilities (n=31) were sequenced with Illumina MiSeq technology. Paired-end fastq files were input into tools for phenotype prediction (ResFinder v.4.4.2; VirulenceFinder v.2.0.3) and typing (MLST v.2.0.9; PlasmidFinder v.2.0.1; NDTree v.1.2). Reads were assembled with SPAdes 3.15.3. The presence of stress-survival islet 1(SSI-1) and serovar-typing was performed with NCBI megablast searches from the contigs generated.

Results: Isolates from food were split 45% (57/128) and 55% (71/128) between lineages I and II, respectively; each lineage contained 20 discrete single nucleotide polymorphism groups. Serovars 1/2b and 3b were indistinguishable and made up 84% (26/31) of the typable lineage I food isolates; serovar 4b represented the remaining five lineage I food isolates. Serovar 1/2a accounted for 92% (55/60) of the typable lineage II food isolates. In food isolates, traits more associated with lineage II when compared to lineage I were stress tolerance genes (52/71 vs. 33/57), SSI-1 presence (59/71 vs. 39/57), *inlA* truncation and/or *inlb* deletion (44/71 vs. 25/57), and antimicrobial resistance genes (43/71 vs. 33/57). Gene *qacG* was observed in five lineage II food isolates. The environmental isolates were split 48% (15/31) serovar 1/2a lineage II and 52% (16/31) serovar-indeterminate lineage I; no stress tolerance genes, antimicrobial resistance genes, or attenuated virulence markers were detected.

Significance: Listeria monocytogenes has higher mortality than most other common foodborne pathogens. Genomic analysis of historic isolates could provide insight into the adaptations L. monocytogenes has made to our food system and suggest mitigation strategies.

P2-241 Resistome, Mobilome, Virulome Analysis and Phylogenomics of *Enterococcus faecalis* Isolated from Raw Muscle Foods of Beef Origin in Gauteng, South Africa

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❖ ***Developing Scientist Entrant***

Introduction: *Enterococcus faecalis* is a widespread bacterium present in diverse environments, including processed beef meat, with serious implications for food safety and public health. This pivotal significance stems not solely from its virulence but also from its adeptness in eliciting multidrug-resistant infections in humans.

Purpose: The aim of this study was to investigate the population structure, resistome, mobilome, and virulome of *E. faecalis* obtained from processed

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beef meat sources in South Africa.

Methods: A total of eight genomes sequenced in this study were examined, alongside 78 publicly available, high-quality genomes of *E. faecalis*, with a comprehensive analysis conducted to identify antimicrobial resistance (AMR) determinants, virulence factors, and mobile genetic elements (MGE).

Results: Six distinct sequence types (STs) (ST79, ST860, ST40, ST238, ST21, and ST700) and 41 core virulence factors were found across all the genomes. The virulence factors included genes encoding adherence (*ace, asa1, Ef0485, ebpA, ebpB, ebpC, srtC*); exoenzyme (*Ef3023, Ef0818, gelE, sprE*); immunomodulation (*cpsA, cpsB, cpsC, cpsD, cpsE, cpsF, cpsG, cpsH, cpsI, cpsK*), and biofilm formation (b*opD, fsrA, fsrB, fsrC*). In addition, AMR genes were identified across all genomes, which include aminoglycoside resistance (*ant(6)-Ia*), trimethoprim resistance (*dfrA*), drug and biocide resistance (*efrA* and *efrB*), multidrug efflux pump (*emeA*), clindamycin quinupristin-dalfopristin, dalfopristin resistance (*lsaA*), and tetracycline resistance (*tetM*). The genomes of *E. faecalis* sequenced here contained a variety of MGEs, including Insertion Sequences (ISs), transposons, prophages, and plasmids, which may have facilitated genetic exchange within and between these species.

Significance: The results highlight that beef meat products act as a reservoir for virulent *E. faecalis* strains possessing antibiotic-resistance traits.

P2-242 Diversity of Environmental and Clinical Strains of *Vibrio parahaemolyticus* in British Columbia

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Introduction: *Vibrio parahaemolyticus* is the leading cause of seafood-associated illness in British Columbia (BC) and yet very little detail exists on the characteristics of *V. parahaemolyticus* strains circulating over time in the province.

Purpose: This study is the first large scale exploration of the genomic diversity of *V. parahaemolyticus* in BC.

Methods: 532 *V. parahaemolyticus* isolates (299 clinical source, 233 oyster source) were selected from a collection of BC isolates spanning 1999 to 2022, balancing years, geography, and known *tdh/trh* gene profiles (as determined by PCR). Isolates were subjected to whole genome sequencing using Illumina platforms and data analysis was completed using a custom bioinformatics pipeline. Analyses conducted included *in silico* MLST and virulence factor gene identification, using the MLST and Abricate tools, respectively. A chi-squared test was performed on each detected gene to identify those that significantly differ between clinical and environmental isolates.

Results: There were 28 sequence types (STs) identified from 299 clinical isolates, of which 5 STs represented 91% of the set. ST36 was most predominant (156 isolates, 55%), followed by ST417 (54 isolates,18%), ST43 (26 isolates, 9%), ST631 (16 isolates, 5%) and ST3 (11 isolates 4%). The remaining 23 STs were observed in ≤ 3 isolates. Conversely, the isolates from 233 oysters represented 78 STs and the 5 STs predominant in the clinical isolates were found in very low levels: ST36 (4 isolates, 2%), ST417 (1 isolate, 0.4%), ST43 (8 isolates, 3%), ST3 (2 isolates, 1%), and ST631 (0 isolates). Multiple virulence factor genes were found in a significantly higher proportion in clinical isolates, including *tdh, trh, vcrV, VopB, VP1611,* and *VopQ* (p <0.001).

Significance: This study forms a baseline genomic understanding of *V. parahaemolyticus* in BC and showcases the marked differences observed in clinical isolates as compared to those found in oysters. The reasons for these differences remain to be explored.

P2-243 Mechanisms of Polymyxin Resistance in Acid-Adapted *Escherichia coli* NCCP 13719 Revealed by Transcriptomics

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Introduction: Microorganisms that acquire acid-adaptation have been found to be resistant to other stresses such as antimicrobials as a result of cross-protection.

Purpose: This study investigates the increased resistance to polymyxins and the mechanisms of resistance to polymyxins by identifying gene expression changes in an acid-adapted *E. coli* strain.

Methods: Acid adaptation in a rich medium (AAR), such as tryptic soy broth, involves conditioning the *E. coli* NCCP 13719 strain (enteroinvasive *E. coli*) to acidic conditions by initially exposing it to a low pH environment. Subsequently, the surviving bacteria were cultured in a neutral pH medium before being subjected to further pH reductions until there were no surviving colonies. Furthermore, antimicrobial resistance profile of the strain was analyzed and transcriptomic analysis was conducted to compare the gene expression profiles between the AAR and control.

Results: Acid-adaptation resulted in a significant increase of resistance to polymyxins. Specifically, AAR exhibited 4-fold increase in the minimum inhibitory concentration of colistin. RNA sequencing analysis showed significant transcriptomic reconfiguration in acid-adapted strains compared to the controls. Numerous genes involved in acid resistance, membrane remodeling, and polymyxin resistance were also found to be upregulated. Genes that respond to acid stress, such as *gadE*, *hdeABD*, and *mdtEF* were highly overexpressed, and contributed to improved acid resistance. In addition, the expression of the outer membrane protein gene *ompX*, which mainly regulates membrane permeability, was increased in the strain. Functional analysis of the acid-adapted strains confirmed a decrease in the outer membrane permeability indicating membrane alterations. The *arn* operon, related to polymyxin resistance, was also found to be upregulated. Transcriptome analysis revealed a significant upregulation of the acid-fitness island, *arnCTE*, and *mdtIJ* genes in acid-adapted strain.

Significance: These results are useful to improve our understanding of antimicrobial resistance and to develop effective strategies to combat the spread of polymyxin resistance.

P2-244 Characterization of Diarrheagenic *Escherichia coli* Isolated from Poultry in the Chobe Region of Botswana by Molecular Methods Including Whole Genome Sequencing

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Introduction: Diarrheal illness incidence is high in rural dryland environments, including the Chobe region of Botswana, where peaks are often associated with high rainfall events. Antibiotic resistance (ABR) is also a growing problem in the region. Contributions of poultry to diarrheal illness and ABR in the region are not well understood.

Purpose: Characterize the virulence potential and ABR of *E. coli* isolated from chicken purchased from local vendors in Botswana using molecular techniques, including whole genome sequencing.

Methods: Poultry (n=61) was purchased in Kasane, Botswana and 100g enriched within 24h. *E. coli* was isolated using MacConkey and EMB agar. Isolates were shipped to Virginia Tech for molecular and antibiotic resistance characterization. Multiplex PCR was used to detect presence of genes *phoA* (all *E. coli),* and diarrheagenic *E. coli (eae,* and *est1b).* Phenotypic ABR was determined using CLSI disk diffusion methods. Whole genome sequencing was performed using an Illumina MiSeq system and genome annotated using BV-BRC.

Results: *E. coli* was isolated from 62% (38/61) of poultry samples. Five isolates were classified as diarrheagenic based on *eae,* none were positive for *est1LB*. Multidrug resistance (3+) was seen in 26 isolates and resistance to 1 or more antibiotics in 41 *E. coli* isolates. Resistance amongst chicken isolates was as follows: tetracycline (29), doxycycline (21), sulfamethoxazole (20), streptomycin (15), ampicillin (15), chloramphenicol (9), ciprofloxacin (6), gentamicin (4), ceftriaxone (4), amoxicillin-clavulanic acid (3) and azithromycin (1). Comprehensive analysis of the novel genomes from chicken intestinal isolates revealed large numbers of virulence genes (125-252) common amongst diarrheagenic *E. coli*. Antibiotic resistance genes (65-85) were also present including those encoding resistance to ampicillin, ceftriaxone, doxycycline, streptomycin, and tetracycline.

Significance: While prevalence of diarrheagenic *E. coli* was low, antibiotic resistant *E. coli* were frequently isolated. *E. coli* may be an important sentinel

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species to evaluate ABR emergence and effectiveness of control strategies by Botswana poultry producers.

P2-245 Evaluation of Metagenomic Wastewater-Based Epidemiology for Enteric Pathogen Surveillance at a Wastewater Treatment Facility

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Introduction: Wastewater based epidemiology (WBE) is a non-invasive surveillance method that has become a routine public health monitoring tool for detecting and tracking emerging pathogens at a community level.

Purpose: Our objective was to evaluate a metagenomic approach for profiling enteric pathogens in wastewater throughout processing as a potential early warning system for scoping outbreaks and detecting the emergence of antibiotic resistance (AMR).

Methods: Raw wastewater samples and 24 hr composites were collected daily in Maryland, USA, January–July 2022. Samples were also collected at points along the treatment process from November 2021 through February 2022. Microeukaryotes were concentrated using Ceres Nanotrap particles. Shotgun metagenomics sequencing was performed using Illumina DNA Prep and NextSeq2000. Data were analyzed using Kraken2, customized bacterial kmer tool, taxaTarget, CARD DB, AMRfinderplus, and AMRplusplus pipelines.

Results: Shotgun metagenomic analyses of 317 samples identified multiple antibiotic resistant *Escherichia coli*, *Salmonella enterica*, *Vibrio* and ESKAPE pathogens. In addition, *Cryptosporidium* and *Giardia* were detected from composite samples. ESKAPE pathogens were not generally detected in samples collected after additional purification of the influent. Temporal trends were observed in the relative abundance of *E. coli*, which increased in late April. Relative AMR abundance per antimicrobial class, such as Oxazolidinone, was generally higher January–March than in the following months. Diversity of species with >5% relative abundance decreased throughout wastewater treatment, while diversity in antimicrobial resistance mechanisms was lowest in purification influent, with increased diversity before and after. Relative proportions of drug resistance decreased whereas metal resistance increased throughout treatment.

Significance: WBE and metagenomic sequencing have the potential to make culture-independent pathogen and AMR surveillance a reality. From a clinical perspective, the metagenomic-level resolution could substantially advance our understanding of the prevalence and scope of foodborne disease outbreaks within specific regions and communities.

P2-246 Survival and Stress Response of *E. coli* O157:H7 during Heat Treatment after Pre-Exposure to Acidic Abomasal Content of Cattle

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7 in healthy cattle has been shown to exhibit adaptation with high acidity of abomasum which may affect its response to post-harvest stress conditions.

Purpose: This study examined the survival and stress-resistant gene expression of *E. coli* O157:H7 after exposure to low pH abomasal content of cattle and subsequent heat treatment.

Method: Abomasum collected from grain-fed cattle was autoclaved, pH adjusted to 5, 4, and 3 using 0.1N HCl, inoculated with *E. coli* O157:H7, and incubated at 39°C for 16h. Post incubation, bacteria were subjected to heat treatment at 55°C and 60°C for 0 to 10 minutes and enumerated using TSA and CT-SMAC plates. Expression of stress-resistant genes (*rpoS*, *rpoH*, *clpB*, and *dnaK*) was analyzed post-low pH exposure and heat treatments (up to 5 min) using RT-PCR and the 2^-ΔΔCT method. The survival data obtained was analyzed using ANOVA and Tukey test for statistical significance ($p<0.05$).

Results: At pH5, all bacteria demonstrated adaptation after 16h of exposure, whereas only ~38% of total bacteria adapted under pH4 conditions. *E. coli* O157:H7 pre-exposed to pH5 exhibited a 2.1 log CFU/mL reduction at 55°C over 10 minutes, whereas bacteria pre-exposed to pH4 showed a higher reduction (3.88 log CFU/mL). When increasing temperature to 60°C the bacteria pre-exposed to pH4 and pH5 survived up to 6 and 4 minutes respectively. *E. coli* O157:H7 was not detected after incubation for 16h at pH3, Heat-resistant gene *rpoH* was significantly upregulated in all treatments, with the highest in pH4 and subsequent heat treatment at 60°C at 0 minute, with up to a 57-fold increase. Gene *clpB* was upregulated after heat treatments with pre-exposure to pH4 and pH5, while *dnaK* also displayed increased expression in all pH5 pre-exposed bacterial heat treatments.

Significance: The study showed that pre-exposure of *E. coli* O157:H7 to acidic conditions can influence its thermal tolerance and response to stress conditions.

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P2-247 Genomic Snapshot of Multidrug-Resistant Environmental *Escherichia coli* and the Relevance in Food Safety

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli*, both commensal and pathogenic, can colonize and persist in various niches. It is used as an indicator of fecal contamination in water quality and food safety monitoring, as well as an indicator of antimicrobial resistance dynamics in a One Health context.

Purpose: The study aimed to evaluate the genomic profiles of 61 isolated ESBL producing *E. coli* from water and fresh produce samples from formal and informal fresh produce production systems in South Africa, using whole genome sequencing (WGS).

Methods: ESBL-producing *E. coli* were isolated from formal and informal fresh produce production systems through selective enrichment, plating onto chromogenic media, identity confirmation using MALDI-ToF analysis, and ESBL production confirmed through double-disk diffusion method. The selected 61 isolates were subjected to WGS (Illumina MiSeq). Antimicrobial resistance genes, virulence genes, plasmid typing, detection of mobile genetic elements, multilocus sequence typing (MLST) and pathogenicity prediction of the strains were determined using Galaxy and the Centre for Genomic Epidemiology (CGE) platform.

Results: A total of 19 known MLST groups were detected among the 61 isolates. Phylogroup B1 (ST-58) and Phylogroup E (ST-9583) were the most common sequence types detected from the water and fresh produce samples. The O101:H9-ST10 strains did not harbor any Shiga-toxin-associated virulence genes however, antimicrobial resistance genes from at least eight different classes were present. Overall, 95.1% of the isolates carried resistance genes from three or more antibiotic classes The $bla_{\text{CTX-M-1,}}$ $bla_{\text{CTX-M-14,}}$ and $bla_{\text{CTX-M-15}}$ ESBL genes were associated with mobile genetics and all of the *E. coli* isolates showed a >90% probability of being a human pathogen.

Significance: This study presented novel genomic information of multidrug-resistant environmental *E. coli* isolates from fresh produce and irrigation water sources in South Africa.

P2-248 *Enterobacter* Isolates Harboring Class A Carbapenemase Genes *bla*NMC-A or *bla*IMI on Chromosomal Integrative Element and Plasmid

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Introduction: Carbapenems are the last-resort for the treatment of multidrug-resistant bacterial infections. Carbapenem resistance poses an escalating threat to global public health. Food products, including fresh vegetables, may serve as reservoirs for antibiotic-resistant bacteria.

Purpose: The study aimed to isolate and characterize six carbapenem-resistant *Enterobacter* isolates obtained from retail vegetable samples.

Methods: *Enterobacter* strains were isolated from 400 retail vegetable samples (lettuce, spinach, carrots, microgreens, and salads) collected in Arkansas using mSuperCARBA CHROMagar after enrichment. Carbapenem resistance phenotypes were confirmed using disk diffusion assay according to the Clinical and Laboratory Standards Institute protocols. Carbapenemase production and typing were determined using the mCIM/eCIM assays. *Enterobacter* genomes were sequenced using an Illumina NovaSeq 6000 sequencer. Bacterial species taxonomy was identified by Ribosomal Multilocus Sequence Typing (rMLST). MLST was carried out using the PubMLST program. Antibiotic resistance genes were identified using the Resistance Gene Identifier (RGI) program against the Comprehensive Antibiotic Resistance Database (CARD).

Results: Six carbapenem-resistant *Enterobacter* isolates were obtained from 6 vegetable samples. Specifically, *E. cloacae* C46, *E. asburiae* C52, and *E. vonholyi* C78 were isolated from three microgreens products originated from the same produce farm. *E. ludwigii* C58 and *E. asburiae* C88 were isolated from microgreens sourced from another farm. Additionally, *E. ludwigii* C1 was isolated from a carrot sample. All six *Enterobacter* isolates produced serine-type carbapenemases and belonged to different MLST sequence types. Notably, *E. asburiae* C52 harbored $bla_{\text{IMI-6}}$ which is likely located on a conjugative plasmid. In contrast, the remaining five *Enterobacter* isolates possessed either $bla_{\text{IMI-1}}$ or $bla_{\text{NMC-A}}$ on chromosomal integrative mobile elements.

Significance: *Enterobacter* is of clinical significance and has been frequently isolated from patients. This is the first report about the isolation of an IMI-6-producing *Enterobacter* from a fresh vegetable. Carbapenem-resistant *Enterobacter* with transferable resistance genes from vegetables may pose a potential risk to human health. This study underscored the importance of monitoring antibiotic resistance in vegetables and production environment.

P2-249 Utilizing Whole Genome Sequencing to Characterize *Listeria monocytogenes* Transmission between a Farmstead Dairy Processing Facility and Its Associated Farm Environment

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❖ ***Developing Scientist Entrant***

Introduction: Farmstead dairy processing facilities may be particularly susceptible to *Listeria monocytogenes* contamination given the close physical proximity of the processing environment (PE) to its associated dairy farm environment (FE).

Purpose: To use whole genome sequencing (WGS) data to characterize the genetic relatedness of *L. monocytogenes* isolates collected from a farmstead dairy processing facility's PE and its associated FE over ~1 year.

Methods: Over the ~1-year study period, environmental sponge samples from the PE (n=120), and raw milk and milk filter sock samples from the FE (n=36), were collected and processed according to the FDA-BAM method for detection of *L. monocytogenes* in environmental/food samples. WGS was performed on select *L. monocytogenes* isolates obtained from the PE (n=15) and FE (n=20) using the Illumina NextSeq 500 platform, and genomes were assembled using SPAdes. Relatedness of genomes was assessed through high-quality single nucleotide polymorphism (hqSNP) analysis using the Center for Food Safety and Applied Nutrition (CFSAN)-SNP pipeline, and clonal complexes (CC) were assigned based on *in silico* multilocus sequence typing (MLST).

Results: FE samples yielded significantly higher *L. monocytogenes* percent positives (n=16, 44%) compared to PE samples (n=14, 12%) ($p<0.05$). hqSNP analysis revealed that 15 *L. monocytogenes* isolates collected from the PE (n=10) and FE (n=5) were closely related (i.e., ≤20 hqSNP differences), suggesting transmission between the PE and FE. Furthermore, these 15 isolates represented CC666, a CC commonly isolated from dairy farm environments, which may support that the FE represented an upstream source of CC666 contamination into the PE.

Significance: Our findings both (i) highlight that the FE can represent a high-risk source of *L. monocytogenes* contamination into a farmstead dairy processing facility's PE and (ii) illustrate how WGS data can be valuably applied towards source tracking and root cause analysis efforts in food processing facilities.

P2-250 Assessment of Method Providers to Detect Artificially Contaminated Microbiota in Blinded Soy Milk Samples

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Introduction: Metagenomics including amplicon-based sequencing are being proposed as routine services by several commercial laboratories to detect the microbiota present in food and environmental samples, however, the lack of standardized methods, availability of numerous DNA extraction, sequencing, and bioinformatics approaches coupled with rapid analytical developments hinder the identification of proficient laboratories.

Purpose: (i) Develop a protocol to assess laboratories proficiency to identify microbiota spiked in soy milk and (ii) Conduct an inter-laboratory trial to identify competent laboratories.

Methods: Samples were generated by spiking commercially sterile soy milk with known concentration of bacteria and fungi. Full length 16S rRNA and ITS gene sequencing using PacBio HiFi was done on DNA extracted by two operators on three different days to generate reference data. Five blinded samples, namely, three spiked replicates and one unspiked soy milk, along with one buffer spiked sample were sent to 6 laboratories to identify the microbiota using their preferred method.

Results: Irrespective of the differences in the methodology, most of the laboratories identified the highest abundant organisms. One laboratory was excluded from the final evaluation as they failed the control sample. Variations in bacterial and fungal relative abundance profiles between three spiked replicate samples were noted for some laboratories, indicating a lack of consistency. The weighted average score measuring the global performance of the laboratories considering detection, relative abundance and proximity to reference profile ranged from 36 to 85/ 100 which allowed the ranking of the laboratories based on their proficiency.

Significance: Differences in the methods applied by the laboratories largely impacted the relative abundance profiles over the identification of the targets. The results highlight the need for a standardized protocol and in the interim the statistical approach applied in this study can be considered for the evaluation of laboratories.

P2-251 Genomic Characterization of an Un-typable Atypical Salmonella spp. Isolated from Mussels in Spain

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Introduction: Detection and identification of atypical *Salmonella* spp. strains represent a challenge for classical methods. This is the case for hydrogen sulfide (H2S) production-negative and *Salmonella* spp. strains.

Purpose: Genotypic and phenotypic characterization of an atypical H_2S production-negative wildtype *Salmonella* strain isolated from farmed mussels in Galicia, Spain.

Methods: Cooked mussels were mixed with BPW and pre-enriched at 37°C overnight. The DNA was extracted with PrepSEQ and analyzed with MicroSEQ *Salmonella* spp. Positive samples were plated on XLD and Brilliance *Salmonella* agar. A pure culture was submitted to a reference laboratory for serotyping and a fresh culture was prepared in BHI at 37°C/150rpm/6h. The DNA was extracted with Purelink Genomic DNA mini-Kit and it was sequenced for 48h in a MinION device (R9.4.1_flowcell), the library was prepared using the Rapid barcoding kit. The *de novo* assembly of the genome was performed using Flye v.2.9 using default parameters and was polished using Medaka. *In silico* MLST, serotyping and antimicrobial resistance detection were performed. The *rfbD* operon was located with Mauve.

Results: H_2S-negative colonies on XLD, but mauve color on Brilliance Agar were observed from qPCR-positive samples. Isolates were non-typable by antisera serotyping. However, *in silico* serotyping identified the strain as *S.* serotype Senftenberg and confirmed by MLST. An inversion of a fragment of the chromosome at the *rfb*D operon was observed, both ends had the same insertion sequence (IS5-like element ISEc68 family transposase) truncating the dTDP-4-dehydrorhamnose reductase gene and separating the reminder genes on the *rfbD* operon. This could be responsible of the absence of detection of somatic antigens. *In silico* AMR only detected the aac(6')-Iaa resistance gene.

Significance: The identification of SH2-negative strains in mussels from Galicia, Spain, highlights the importance of molecular methods, not affected by the phenotype, to assure the innocuousness of this product, and assuring food safety.

P2-252 Microbiota and Population Dynamics During Selective Enrichment of *Listeria monocytogenes* in Drains From a Sausage Processing Plant

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Introduction: Subtyping of *Listeria monocytogenes* from food processing plants for the purpose of source tracking, e.g. using WGS analysis, is usually performed on a single colony obtained after selective enrichment.

Purpose: To study the diversity of *L. monocytogenes* and evaluate the impact of endogenous microbiota on the population dynamics during selective enrichment of samples from drains at a sausage processing plant.

Methods: Six different drains in the processing plant were each sampled four times over a period of eight weeks and analyzed for *L. monocytogenes* according to ISO 11290-1 (enrichment in half Fraser and Fraser broth). Counts of *L. monocytogenes* and the total viable count were determined at inoculation (0 hours) and after 4, 24, and 48 hours of enrichment. Samples from each time-point were subjected to microbiota analysis using 16S rRNA amplicon sequencing and (for one of the enrichment cultures) shotgun sequencing. The STs of up to 10 single *L. monocytogenes* colonies (total 460 isolates) from each *L. monocytogenes*-positive sample were determined using a real-time PCR assay or WGS.

Results: Three drains were positive for *L. monocytogenes* on all four sampling occasions, two were positive on three out of four samplings, while one was only positive on the first sampling occasion. No change in the initial microbiota composition was seen after 4 hours of enrichment. The most commonly identified genera were *Pseudomonas*, *Acinetobacter*, *Janthinobacterium*, *Chryseobacterium*, *Staphylococcus*, and *Sphingomonas*. There were no apparent differences in the microbial genera present in *L. monocytogenes* positive and negative drains or samples. Four different *L. monocytogenes* STs were identified – ST8, ST9, ST121, and ST451 – with up to three different STs present in the same sample.

Significance: Subtyping of a single *L. monocytogenes* colony after enrichment may result in loss of diversity and complicate source tracking efforts, as many different *L. monocytogenes* subtypes may be present in the same sample.

P2-253 A Bioinformatic Approach to Identify Targets for Detection and Genotyping for a *Cronobacter* Targeted Amplicon Sequencing Assay

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Introduction: *Cronobacter sakazakii* causes dangerous infections to infants under 2 months of age after ingestion of contaminated powdered infant formula (PIF). *C. malonaticus*, and *C. turicensis* also have the potential to cause foodborne infections.

Purpose: A targeted amplicon sequencing assay (TAS) was developed for detection and typing of three *Cronobacter* species from PIF sample matrices.

Methods: 152 representative whole genome sequences from *Cronobacter* and related genera were downloaded from NCBI. 3646 annotated genes of *C. sakazakii* ATCC BAA-894 were aligned with their BLAST matches in the representative genomes. Target regions were identified in each alignment that had a variable region for strain-level discrimination and species-level taxonomic differentiation and had conserved flanking regions for primer design. A Shannon entropy of at least 2.0 was set for clear distinction of species in each target. An internal database with homologous sequences from 100+ sequence types of *Cronobacter* and near-neighbor genera within *Enterobacteriaceae* was developed for validating species specificity and strain-level variation and designing primers for wet-laboratory method optimization and testing using PIF sample matrices.

Results: For *C. sakazakii,* 55 target regions of appropriate amplicon size were found to be present in all representatives of that species with variation in the amplicon for typing and with conserved flanking ends. A phylogenetic tree based on the 55 target regions in 56 representative *C. sakazakii* strains clearly separated the sequence types into separate clades. From *C. malonaticus* and *C. turicensis* gene alignments, 59 and 57 targets respectively were selected from several hundred possible targets, ranked on variation in the amplicon allowing distinction from other species.

Significance: This *Cronobacter* TAS assay will enhance laboratory capability for rapid species-identification of PIF contaminants. In conjunction with qPCR-based surveillance efforts, the accurate identification and high-resolution genotyping obtained from TAS will provide critical insight for PIF safety, surveillance, and regulatory practices.

Poster

P2-254 Whole-Genome Sequencing of *Salmonella* Isolated from a Vegetable Supply Chains in Cambodia Revealed a High Serovar Diversity and Signs of Persistence and Transmission in the Supply Chain

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Introduction: The specific stages in Cambodian vegetable supply chains where contamination persists remain unclear, therefore, improved understanding of pathogen transmission and persistence is critical for improving food safety and implementing evidence-based control measures.

Purpose: This study investigated genomic similarity and persistence of *Salmonella* isolates across three stages of a vegetable supply chain.

Methods: *Salmonella* was confirmed using selective/differential media followed by *invA* PCR. Confirmed isolates were whole-genome sequenced, assembled using MicroRunQC, and those that did not meet the GenomeTrakr surveillance network assembly metrics were removed from the study. Serovar diversity was assessed using SISTR. Phylogenetic diversity was evaluated by building a maximum likelihood tree using core genome single nucleotide polymorphisms identified with kSNP4 (all isolates) and CFSAN SNP pipeline (serovar groups).

Results: Out of 78 *S. enterica* isolates, twenty-nine different serovars were detected. The most abundant were Paratyphi B Java, Hvittingfoss, and Thompson (n=14, n=11, n=7, respectively). Paratyphi B Java was mostly found in farms (n=5) and markets (n=6), Hvittingfoss was abundant within distribution centers (n=8), and Thompson was found at markets (n=4) and farms (n=3). Within Paratyphi B Java, one phylogenetic clade contained four closely related isolates (0-1 SNP difference) collected at markets in different provinces on different days. Another clade contained two isolates that differed by one SNP. One of these isolates was obtained from a Battambang farm, and the other one from a Siem Reap distribution center. Hvittingfoss isolates clustered in two clades. One clade contained five identical Battambang isolates, four of which were obtained from the distribution center and one from a farm, in different months. Another clade contained three isolates from the Battambang distribution center that differed by 0-1 SNPs.

Significance: These findings suggest a broad spread of Paratyphi B Java in the Cambodian vegetable supply chain and possible transmission of *Salmonella* between stages.

P2-255 Molecular Characterization of Non-O157 STEC *Escherichia coli* Isolated from Western Canadian Cattle

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Introduction: Shiga toxin-producing *Escherichia coli* (STEC) pose a substantial threat to public health, with non-O157 serotypes increasingly recognized as significant contributors to foodborne outbreaks.

Purpose: The molecular characterization of non-O157 STEC serotypes isolated between 2013 and 2015 from Western Canadian cattle was conducted, elucidating their genomic diversity and virulence profiles.

Methods: Thirty-one non O157 STEC isolates (serogroups O26, O45, O103, O111, O121, and O145) from feces of harvested cattle, were subjected to whole-genome sequencing (WGS). DNA was extracted with the Qiagen DNeasy Kit, and libraries were prepared using the Illumina DNA LP (M) Tagmentation kit. WGS was performed on the Illumina MiniSeq (300 cycles). Raw reads were filtered, trimmed, assembled, and analyzed using bioinformatic tools.

Results: Multi Locus Sequence Typing classified the 31 isolates into 15 distinct Sequence Types (STs). ST-16 was the most common observed and predominated among isolates from serotypes O45:H4 and O103:H2. Serotype O111:H8 isolates carried the most virulence genes (141/201), followed by O26:H11 with more than 100 virulence genes. Thirteen of the 31 isolates (41.9%) were positive for *stx* genes. All O111:H8 isolates were *stx1* and *eae* positive, whereas the gene *stx2a*, strongly associated with severe disease, was found only in serotypes O121:H19 and O145:H-. A total of 58 AMR genes were detected *in silico*, and each isolate carried more than 40 AMR genes, of which O111:H8 isolates carried the most, including genes for sulfonamide (*sul*2), tetracycline (*tet*), and aminoglycoside (*aph*).

Significance: The findings of this study highlight the complexity of non-O157 STEC populations in cattle and their implications for food safety. Enhanced understanding of the genotypic and phenotypic characteristics of these foodborne pathogens informs targeted surveillance and intervention strategies, aiming to reduce the incidence of foodborne outbreaks associated with non-O157 STEC.

P2-256 Cronology: An Automated Bioinformatics Workflow for *Cronobacter* Whole Genome Sequence Assembly, Subtyping and Isolate Clustering

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Introduction: *Cronobacter* genus comprises seven species, one of which is *C. sakazakii*, an opportunistic pathogen found in foods including powdered infant formula, other environments such as soil, and insects.

Purpose*:* To develop an automated and accurate data analysis workflow for *Cronobacter* species identification and subtyping from whole genome sequence data.

Methods*:* Cronology includes a custom database and a bioinformatics workflow written in Nextflow. The database is composed of high-quality, diverse genomes spanning all seven *Cronobacter* species. During the custom database generation, MLST is executed using the PubMLST *Cronobacter* spp. schema on quality filtered genomes and the results are indexed. The cronology workflow ingests raw Illumina reads (single-end or paired-end) and performs read quality filtering using fastp. *De novo* genome assembly and polishing are performed with Shovill and polypolish. Users have the option to supply a reference genome accession for gene prediction and annotation using prokka. Assembly metrics are generated with QUAST, and species identification is predicted using ribosomal MLST (RMLST). Clustering and phylogenetic analysis is performed for each isolate with mash and mashtree, producing newick formatted tree files with metadata that can be visualized on iTOL. A brief HTML report for each experiment is generated using MultiQC. Cronology was benchmarked using publicly available *Cronobacter* genomes plus an in-house dataset isolated from insects. The cronology workflow is publicly available at https://github.com/CFSAN-Biostatistics/cronology and on CFSAN GalaxyTrakr.

Results*: Cronobacter* isolate sequences (n=627) were analyzed using cronology. Taxonomy was accurately predicted for each of the sequences and MLST analyses revealed novel sequence types. *C. dublinensis, C. malanoticus, and C. turicensis* were all correctly identified and typed along with *C. sakazakii*. *Cronobacter* isolates (n=16) from insects, were correctly typed, and identified, and they clustered with strains from insect alimentary canals.

Significance: These analyses achieved precise species taxonomy, subtyping and isolate clustering for *Cronobacter* spp.

P2-257 Serovar Identification, Predicted Antimicrobial Resistance, and Clinical Relevance of *Salmonella enterica* Collected from Chicks Shipments Destined for the Hobby Poultry Industry

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Introduction: Chicks sold to the hobby poultry industry have been linked to salmonellosis cases in purchasers over the past decade.
Purpose: To determine the clinical significance and antimicrobial resistance of *Salmonella enterica* isolates previously collected from hatchling chick shipments sold at agricultural supply stores in Vermont.
Methods: The Vermont Department of Health performed Illumina MiSeq sequencing (paired end; Nextera DNA Flex library preparation) for us on 42 *S. enterica* isolates we collected in 2021-2022. These were assessed for predicted serotype, single nucleotide polymorphism (SNP)-based clustering with clinical cases, and antimicrobial resistance (AMR) genes using NCBI's pathogen detection system.
Results: Most isolates (22; 52.4%) were serovar Enteritidis; eight (19.1%) were serovar Infantis, two isolates each were serovars Hadar, Kentucky, Seftenburg, Mbdanka, and Cerro, and one each was serovar Liverpool and Ouakum. *Salmonella* Kentucky was only isolated from meat chickens. *Salmonella* Enteritidis was primarily isolated from laying chicken breeds and mapped to two SNP clusters, PDS000032668.1100 (14 isolates) and PDS000032668.1105 (7 isolates), with 0-5 SNPs between our isolates and the nearest clinical isolate. Serovar Infantis was isolated from ducks (1), turkeys (2), and laying chicken breeds (5), and clustered primarily in SNP cluster PDS000027076.764 (0-4 SNPs to nearest clinical isolate). Isolates from serovars Enteritidis, Infantis, Cerro, Mbdanka, or Seftenburg carried no AMR genes. *Salmonella* Hadar isolates encoded resistance to aminoglycosides and tetracycline (*aph(6)-Id* and *tet(A)*), as well as *gyrA*_S83Y mutations encoding fluoroquinalone resistance. The Liverpool isolate carried genes for aminoglycoside resistance and tetracycline resistance (*aph(3")-Ib, aph(6)-Id, tet(B)*), as well as the non-functional *mcr-9.1* gene, while the *Salmonella* Oakum isolate encoded resistance to sulfonamides, tetracycline, and aminoglycosides (*sul2, tet(A), aph(3")-Ib, aph(6)-Id*). One *Salmonella* Kentucky isolate encoded resistance to aminoglycosides and tetracycline (*aph(3")-Ib, aph(6)-Id, tet(A)*).
Conclusions: *S. enterica* isolates from chicks sold at agriculture supply stores were closely related to clinical isolates but rarely contained AMR genes.

P2-258 Die-off Rates of *E. coli* O157:H7 in Agricultural Soils

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Introduction: Yuma, Arizona produces 90% of the leafy greens in the United States from mid-November to mid-April, therefore understanding *Escherichia coli* O157:H7 survival in the environment from this growing region is critical for food safety. Studies have shown that *E. coli* O157:H7 can survive in soil for up to 266 days, but these studies were not conducted on Arizona soils or growing conditions.
Purpose: The purpose of this study was to determine the die-off rates of *E. coli* O157:H7 strains in soils directly from Arizona leafy green fields and assess how these rates were impacted by different location conditions (Yuma, AZ versus Maricopa, AZ), soil amendments, and high soil moisture.
Methods: Microcosms with 100g soil from different leafy greens fields from Yuma or Maricopa were established in sterile non-treated tissue culture flasks in triplicate and inoculated with 10^7 CFUs of one of two strains of *E. coli* O157:H7 either strain REPEXH01 (2018 romaine lettuce outbreak) or TW14359 (2006 spinach outbreak). Inoculated soil microcosms were maintained in plant growth chambers to control temperature, humidity and sunlight for local conditions (Maricopa or Yuma). Soil microcosms were cultured/sampled for viable *E. coli* O157:H7 at 7, 10, 14, 21, 30-, 60-, 90-, and 120-days post-inoculation.
Results: Neither *E. coli* O157:H7 strain on average survived longer than 30 days, and there was no significant difference between different soil types (p=0.90) or the presence of soil amendments or moisture (p=0.28). However, there was a significant difference in survivability based on location, as strains survived longer in Yuma soil and conditions than Maricopa (p=0.01).
Significance: This study provides Arizona leafy green growers with an understanding that *E. coli* O157:H7 potentially does not survive for longer than 30 days in fields after a contamination event, but that survivability will vary based on location in Arizona.

P2-259 Rapid Identification and Characterization of Bacterial Foodborne Pathogens through Oxford Nanopore-Based Whole Genome Sequencing

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Introduction: Whole genome sequencing (WGS) has emerged as a pivotal tool for understanding, tracking, and mitigating the impact of foodborne illnesses. Advancements in Oxford Nanopore Technologies' sequencing have significantly improved accuracy for microbial genomic applications, rendering it a promising rapid, cost-effective tool for public health and food safety.
Purpose*:* Using DNA from 32 bacterial isolates sourced from the FDA CFSAN proficiency panel, we evaluated the use of Oxford Nanopore as a standalone platform for accurate whole genome identification and characterization of microbial food contaminants.
Methods*:* Bacterial DNA was prepared with the Rapid Barcoding Kit (1 hr) and sequenced (up to 72 hr) with the latest chemistry and basecalling software in triplicate, with 16 samples multiplexed per run. The resulting nanopore reads were assembled into consensus genomes, antimicrobial resistance (AMR) profiles were determined with AMRFinderPlus, and *Salmonella* strains were serotyped using SeqSero2. Samples were subsampled and reassembled to generate 10x-100x coverage nanopore-only assemblies, and then benchmarked against publicly available reference genomes.
Results: Nanopore sequencing of the 32 panel isolates in triplicate generated 96 genomes with a mean coverage of 166x (or 75x in 24 hr sequencing time), and an N50 of ~6 Kbp. Reference genomes were identified for 27 of the 32 isolates, and technical replicates with >=100x coverage were carried forward for benchmarking (n=72). At 100x coverage, 24% (17/72) samples yielded >=Q60 error-free genomes. At 30x, samples achieved a mean/median base accuracy of Q52 (99.999%), >=Q60 in 15% (11/72) samples, 100% sensitivity/specificity for AMR profiling (72/72 samples), and a 100% match rate for *Salmonella* serotypes (15/15 samples).
Significance: This validation study demonstrates nanopore sequencing as an accurate, rapid, and cost-effective method compared to conventional confirmation testing and traditional sequencing platforms. It has the potential to improve the efficiency of surveillance, tracking, and risk assessment of foodborne pathogens.

P2-260 A Tale of Two Kentuckys: Highlighting Genetic and Phenotypic Differences Within a Polyphyletic *Salmonella* Serovar

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* serovar Kentucky is the top serovar isolated from broilers but is infrequently associated with human salmonellosis in the United States. Elsewhere (e.g. Europe and North Africa), Kentucky has a higher association with human illness. *Salmonella* serovar Kentucky is polyphyletic with two main lineages, Kentucky-I and Kentucky-II, which also have distinct antibiotic resistance profiles.
Purpose: This study sought to assess the relative incidence of *Salmonella* Kentucky-I and Kentucky-II in food animal production and human salmonellosis cases in the United States as well as to assess genetic and phenotypic differences between the two lineages.
Methods: Publicly available sequences (n=8,281) deposited to NCBI by the USDA-FSIS and the CDC between 2017-2023 were analyzed in this study. Genomes were identified as Kentucky-I or Kentucky-II based on their CRISPR content. The virulence factor database was used to assess differences in viru-

lence factors between both lineages. A gentamicin assay with RAW264.7 murine macrophages was used to evaluate association, invasion, and replication in both *Salmonella* Kentucky lineages.

Results: Kentucky-I was the most prevalent serovar Kentucky lineage found in broilers 99.8% (7557/7570), swine 97.7% (43/44), turkey 60.0% (12/20), and bovine 54.6% (125/229). Conversely, Kentucky-II was most prevalent in human clinical isolates 57.2% (239/418). Overall, there was no difference between Kentucky-I and -II with association and invasion in macrophages. The Kentucky-II isolates were able to replicate within macrophages 8.6 times more than Kentucky-I isolates, which differed significantly from Kentucky-I (Tukey-Kramer HSD test). Kentucky-I genomes lacked key virulence genes, including the *tcf* operon and *sseK2.*

Significance: This data highlights the importance of examining genetic differences within a serovar, rather than relying solely on serotyping data, especially for polyphyletic serovars (~10% of *Salmonella* are polyphyletic). Given the association with human illness, Kentucky-II frequency in food animals should continue be closely monitored.

P2-261 Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) in *Campylobacter jejuni* Isolates from Poultry

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Introduction: Bacteriophages have been added in poultry feed as alternative antimicrobials to control *Campylobacter* contamination during production. To make this approach effective, the presence of the bacteriophage gene sequences as the CRISPR spacers in *Campylobacter jejuni* genomes is critical. However, this microorganism often develops resistance mechanisms against invading bacteriophages. CRISPR is one of such mechanisms.

Purpose: In this study, we explored the distribution of CRISPR sequences in *Campylobacter jejuni* isolates from poultry sources.

Methods: A total of 310 *Campylobacter jejuni* isolates were used in this study. Their genomic DNA was extracted using a commercial kit. CRISPR type1 sequences were amplified by PCR according to the standard protocol. Amplicons were purified and sequenced by the Sanger dideoxy sequencing method. Direct repeats and spacers of CRISPR sequences were identified using the CRISPRFinder program. Further, spacer sequences were analyzed with the CRISPRTarget program to identify potential homology to bacteriophage types.

Results: The lengths of the CRISPR sequences ranged from 98 to 695 nucleotides from 310 *Campylobacter jejuni* isolates containing one to 10 spacer sequences. The sequence of the direct repeat was 5'-ATTTTACCATAAAGAAATTTAAAAAGGGACTAAAA-3'. A total of 924 spacer sequences were identified. With alignment by the MUSCLE alignment software, 155 different spacer sequences were obtained. Further analysis with the CRISPRTarget program, the results show these spacer sequences were dominantly homologous (scores 20 – 30) to small fragments of the *Campylobacter* phage DA10 genome, including genes for baseplate J-like protein, tailtape measure protein, major capsid protein, large terminase subunit, site-specific DNA methyltransferase, DNA replication protein, RepO-like protein, anti-repressor protein and 13 hypothetical proteins.

Significance: The results of our study provide important information relative to development of an effective bacteriophage treatment to mitigate *Campylobacter* during poultry production.

P2-262 Untargeted Metabolomics Guided the Discovery of Biomarkers for *Pseudomonas aeruginosa* Hypoxic Biofilm

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Introduction: *Pseudomonas aeruginosa* is a versatile opportunistic pathogen which causes a variety of food spoilage incidents, acute and chronic human infections, many of which are associated with the biofilm phenotype of the pathogen.

Purpose*:* We hypothesize that defining the intracellular metabolome of biofilm cells, compared to that of planktonic cells, will reveal the metabolic pathways and accumulated biomarkers characteristic of biofilm inception.

Methods: *P. aeruginosa* ATCC 9027, a prolific biofilm producer, was grown in Lysogeny broth at 37°C for 24 h with shaking to prepare planktonic growing cells. For biofilm mode of growth, disk-shaped stainless-steel coupons (12.7-mm in diameter) were used. Each disk coupon was immersed in 1-mL of diluted *P. aeruginosa* cell suspension before the microtiter plate was statically incubated at 37 °C for 24h under oxygen-depleted conditions to investigate hypoxic biofilm, an underexplored phenotype. Planktonic and biofilm cells were harvested and resuspended in cold methanol-deionized water mixture containing 0.5-mm glass beads to release the intracellular metabolites which were subject to global untargeted metabolomic analysis using LC-MS technology. A panel of statistical univariate, multivariate, and functional analyses (e.g., KEGG) were implemented on the metabolomic data.

Results: Metabolomic data analysis showed the presence of 324 metabolites that differed ($p < 0.05$) between planktonic and biofilm cells whereas other 70 metabolites that did not vary between the two cell phenotypes ($p > 0.05$). Correlation analysis (Pearson coefficient) and partial least square discriminant analysis illustrated that the biofilm metabolome (metabolites < 1.5 kDa) is distinctly clustered from that of the planktonic cells. Functional enrichment analysis discovered that arginine and proline metabolism were enriched in planktonic cells whereas butanoate metabolism was enriched in biofilm cells. Exogenous supplementation of acetoin (2 mM), a critical metabolite in butanoate metabolism, augmented biofilm mass and maintained the intracellular redox potential.

Significance: The butanoate pathway metabolites, particularly acetoin, could serve as markers for early biofilm detection in the food environment.

P2-263 Adaptive Laboratory Evolution of *Salmonella enterica* Under Prolonged Acid Exposure

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Introduction: Adaptive laboratory evolution (ALE) is crucial for understanding bacterial responses to environmental stresses. This study focuses on the ALE of *Salmonella enterica* serovar Enteritidis (SE) after acetic acid (AA) exposure.

Purpose: The study aims to comprehensively grasp the phenotypic, genomic, and transcriptomic evolution of SE under prolonged acid exposure through ALE.

Methods: AA concentrations below the minimum inhibitory concentrations (MIC) were used to develop four evolutionary lineages (EL1-EL4) of SE. EL1, the control, group, was not exposed to AA. Over a 70-day period growth rates, resistance to human antibiotics, and genomic alterations in the ELs were quantified. A new lineage, EL5, was created to assess the stability of phenotypic changes after 70 days, including genomic and transcriptomic analyses.

Results: EL1 maintained a constant AA MIC of 27mM, while EL2-EL4 exhibited increasing MIC over 70 days. EL4, adapted to 30mM AA, showed the highest AA MIC increase, reaching 35mM on day 70. Growth rate of EL4 increased significantly (0.33 h^{-1}) compared to EL1 (0.2 h^{-1}) after 70 days in AA ($p<0.01$). Long-term AA exposure increased MICs of human antibiotics such as ciprofloxacin and meropenem against the SE lineages EL2-EL4. Ciprofloxacin MIC of EL1 remained constant (0.016ug/ml) over 70 days but increased to 0.047ug/ml for EL4. Whole genome sequencing identified mutations in ELs affecting virulence-associated genes such as *phoP, phoQ, fhuA*. Elevated antibiotic MICs in EL4 after 70 days diminished upon AA stress removal but recovered quickly upon stress reintroduction. AA MIC in EL4 remained stable after AA stress removal and increased significantly ($p<0.01$) upon stress reintroduction. EL5 exhibited base substitutions in virulence genes and transcriptomic analysis showed upregulation of antibiotic-resistance genes such as SugE and pathogenesis-related genes such as SpvA.

Significance: These findings enhance our understanding of bacterial evolution and adaptation under stress and its potential implications for public health.

P2-264 *Salmonella enterica* Serovar Schwarzengrund: Distribution, Virulence and Antimicrobial Resistance

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Introduction: The numbers of *Salmonella enterica* serovar Schwarzengrund infections have been increasing globally in recent years. This serotype has been isolated from both poultry, retail meat and other foods, leading to multiple outbreaks. Alongside the increase in infections, there are concerns about the increasing levels of antimicrobial resistance (AMR) among *S*. Schwarzengrund strains.

Purpose: To better understand the genetic factors possibly contributing to the growing prevalence of *S.* Schwarzengrund, the sequences of 2,058 isolates from human patients (n=313) and food and animal-associated sources including chicken (n=1,145), turkey (n=300), and pork (n=132) were analyzed.

Methods: The data were extracted from GenBank and evaluated for their AMR genes with AMRFinder. Additionally, putative virulence genes and plasmid transfer genes were assessed using the Virulence and AMR Plasmid Transfer Factor Database.

Results: AMR genes were common among the isolates, with 1269 (61.7%) carrying at least one AMR gene. The most commonly observed resistance genes included *aph(3")-Ib* (aminoglycoside; n=969, 47.1%), *tet(A)* (tetracycline; n=190, 9.2%) and *sul2* (sulfonamide; n=150, 7.3%). Among the identified plasmid types, approximately 1,060 (51.5%) carried multiple transfer genes associated with IncFIB-FIC plasmids. Additionally, IncI1 (n=101, 4.9%), IncHI2 (n=62, 3.0%), or IncHI1 (n=24, 1.2%) genes were detected in at least 1% of the strains. The virulence gene profiles of isolates from human patients exhibited diversity, yet they typically overlapped with profiles from different food sources. The aerobactin iron acquisition genes were prevalent among chicken isolates (n=1019, 89.0%), but less frequent among isolates from other sources (n=65, 7.2%).

Significance: IncFIB-FIC plasmids, commonly harboring the aerobactin operon, were highly prevalent among isolates from chicken-related sources and approximately 10% from human patients. Among human patient isolates, diverse virulence gene profiles were observed, suggesting that multiple factors may contribute to increased virulence in *S*. Schwarzengrund.

P2-265 Evaluation of Virulence Factors as Targets for Veterinary Drugs for Avian Pathogenic *Escherichia coli* to Combat Colibacillosis in Chickens

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Introduction: Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is an infectious disease affecting poultry flocks. Although production strategies have been adopted to control the spread of this disease, treatment continues to rely on antimicrobial therapies, potentially contributing to antimicrobial resistance (AMR). By targeting microbial virulence, antivirulence (AV) drugs may be a useful adjunctive or alternative method for treating diseases.

Purpose: Using colibacillosis as a model infection, this project explores the kinds of information and data necessary to support the FDA's Center for Veterinary Medicine's (CVM) pre-approval evaluations of potential AV drugs.

Methods: 769 isolates from clinical APEC cases and 1218 isolates from apparently healthy poultry ceca were evaluated for genetic targets preferentially associated with clinical APEC. AMR, virulence gene, and plasmid identification was accomplished via whole genome sequencing (WGS) analyses using *AMRFinderPlus*, a virulence gene database developed at FDA, and *PlasmidFinder*. Short-read WGS, antimicrobial susceptibility testing (AST), and biofilm assessments were performed on 170 APEC. Correlations between virulence genes, biofilm formation, AMR genotype/phenotype, and plasmid type were made using Principal Component Analysis.

Results: Virulence genes most associated with diseased poultry included *flgB, gsp, csgF, iss*, and iron acquisition factors such as *iutA* and *iucA-D*. AST results showed similarly high levels of ampicillin and tetracycline resistance (43.2% and 59.5%) in isolates from healthy and diseased poultry (48.8% and 71.2%). Based upon this work, there remains uncertainties to address regarding potential co-selection of AMR and bacterial virulence, and how the use of AV drugs may impact the selection of both.

Significance: Alternative therapies, such as AV drugs, can be employed to treat diseases and provide alternatives to antimicrobial treatments. Evaluating data which contribute to the effectiveness of such therapies, including the risk of unintended consequences such as co-selection of antimicrobial resistance, is critical for establishing criteria for CVM's approval pathways.

P2-266 Whole Genome Sequencing and Characterization of *Campylobacter jejuni* Strains S27, S33, and S36 Newly Isolated from Retail Chicken

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Introduction: *Campylobacter jejuni* is a major cause of foodborne gastroenteritis worldwide, mainly associated with the consumption of contaminated poultry products. The molecular mechanism behind *Campylobacter* infection remains incompletely understood suggesting dependance on multiple virulence factors involving cell adhesion, invasion, and motility.

Purpose: The purpose of the study is to examine the genomic characteristics of *C. jejuni* strains (S27, S33, and S36) recently isolated from retail chicken in US, reveal the genetic determinants of virulence and antibiotic resistance, and expand our understanding of the molecular basis of *Campylobacter*-host interactions.

Methods: *Campylobacter* strains were isolated from raw chicken acquired from local supermarkets in 2023 using a passive filtration technique. Raw sequences, *de novo* assembled and annotated genomes of the strains were obtained by using PacBio long and accurate HiFi sequencing technology combined with bioinformatics tools. Comparative genomics study was performed to predict virulence and resistance genes, and mobile genetic elements.

Results: The assembled genomes contain circular chromosomes with sizes 1.6-1.7 Mb and G+C contents 30.4-30.5%. *C. jejuni* S33 harbors a 40.7 kb plasmid (pCjS33), while *C. jejuni* S36 carries an 86.8 kb plasmid (pCjS36). Comparison with available plasmids in GenBank showed that pCjS33 shares 99.30% sequence identity and 96% query coverage with *Campylobacter* pTet plasmid, while pCjS36 exhibits up to 99.87% sequence identity and 98% query coverage with multiple *C. jejuni* plasmids. Functional annotation revealed several virulence and resistance genes in each chromosome, a type IV secretion system in pCjS33, and a type VI secretion system and a phage in pCjS36. The presence of these genes within the genomes could confer advantages to *C. jejuni*, enhancing survival, adaptation, transmission, and pathogenicity across different environments and hosts.

Significance: These findings emphasize the risks associated with *C. jejuni* infection originating from retail chicken and contribute to understanding the molecular mechanisms governing *Campylobacter*-host interactions and horizontal gene transfer, which may facilitate the development of novel therapeutic strategies for managing campylobacteriosis.

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P2-267 Targeted Genomic Sequencing of Noroviruses in Contaminated Oysters

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Introduction: Genomic sequencing of noroviruses (NoVs) in oyster tissues is challenging due to low viral loads and a complex specimen matrix. Consequently, this technology has not been widely used to investigate NoV outbreaks caused by contaminated oysters.

***Purpose*:** We developed and validated a targeted genomic sequencing method for recovering NoV sequences from challenging oyster specimens.

Methods: Our method uses a custom panel of hybridization probes to capture and enrich NoV genomic material in Illumina libraries prepared from oyster tissue specimens. Probe design was based on 51,442 NoV reference sequences to provide broadly inclusive coverage of all genotypes within genogroups GI and GII. We also developed a custom bioinformatic pipeline that identifies genogroup and genotype of recovered NoV sequences. The pipeline also identifies and discards common sequencing artefacts, increasing confidence in weak NoV detections. We validated our method on batches of oysters that were artificially fed NoV-contaminated stool from human patients (n=5). We also assessed our methods on naturally contaminated oysters in which GI and GII NoVs had been previously detected by qPCR-based nucleic acid testing (n=30).

Results: NoV sequences were recovered from 5 of 5 (100%) artificially-fed oyster batches. Genogroups and genotypes of recovered NoV sequences matched the genogroups and genotypes of the NoVs that had been fed to the oysters. NoV sequences were recovered from 23 of 30 (76.7%) naturally contaminated oyster specimens, and the following genotypes were detected: GII.17, GII.3, GII.P12, GII.P16, and GII.P17. Overall, 184 distinct genome fragments were recovered from the naturally contaminated oysters; they were mostly short, but some longer fragments were obtained (range: 102 to 1,005 nucleotides, median: 283 nucleotides).

Significance: Our custom hybridization probe capture-based method is well-suited for the challenges of sequencing NoV genomic material in oyster specimens. This approach could be deployed for genomic sequencing of contaminated oysters and source tracking of NoV outbreaks.

P2-268 A Modern Bioinformatics Pipeline for Norovirus Typing and Phylogenetics

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Introduction: Norovirus is the leading cause of acute gastroenteritis worldwide. The typing of norovirus, which entails sequencing of the capsid gene (genotype) and polymerase gene (p-type), plays an important role in surveillance and outbreak analysis. However, current norovirus typing approaches, which commonly use Sanger sequencing, provide limited resolution and accuracy due to the short contig available for analysis.

Purpose: Here, we present a modern bioinformatics pipeline, along with associated sequence databases, capable of assembling whole genome norovirus sequences and providing high-resolution typing and phylogenetic results using next-generation sequencing (NGS) data.

Methods: We curated two sequence databases based on data from Vinje (2019), one for genotyping and another for p-typing. We developed a custom Nextflow pipeline which performs quality control, typing, de-novo assembly, read mapping, consensus generation, and phylogenetic analyses on clinical norovirus data. Our pipeline has been tested on NGS data generated by a single amplicon protocol previously described by Parra (2017). However, the modularity of Nextflow makes the pipeline adaptable to other formats as needed.

Results: For all 42 available comparisons, this pipeline matched the typing calls of two modern online tools hosted by 1) Center for Disease Control (US), 2) National Institute for Public Health and the Environment (Netherlands). Furthermore, the pipeline correctly called multiple types in experimentally generated mixed samples. For 32 of 45 tested isolates, we assembled a full or near-full genome longer than 5000 nucleotides. When excluding isolates demonstrating amplicon dropout, final consensus sequences had a mean completeness of 92.6%. In phylogenetic trees, all isolates clustered adjacent to the reference sequence matching their typing call, as expected.

Significance: This pipeline enables high-resolution typing and phylogenetic analysis of clinical norovirus isolates and facilitates the future use of NGS methods in norovirus research, surveillance, and outbreak analysis.

P2-269 Genomic Characterization of a *Cronobacter sakazakii* Strain ST64 Recovered from Spice Powder

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Introduction: *Cronobacter sakazakii* is an opportunistic, Gram-negative, foodborne bacterium that can cause life-threatening meningitis and necrotizing enterocolitis in neonates, elderly, and immunocompromised individuals. It can survive in extreme dry conditions and has been linked primarily to contaminated powdered infant formula (PIF). It has also been isolated from a wide variety of foods. Thus far, WGS is recognized as a novel comprehensive technique that has improved bacterial typing. It is now established as the optimal method for typing bacterial pathogen and analyze foodborne outbreaks. Previously, we have identified *C. sakazakii* CC4 and ST21 strains from spice powder samples utilizing the WGS. In this study, we describe the draft genome sequence of a *C. sakazakii* strain SRL-40, recovered from spice powder.

Purpose: The major objective of this study was to identify and characterize a *C. sakazakii* strain recovered from spice powder by performing WGS analysis.

Methods*:* In this study, a *C. sakazakii*-like bacterial strain from spice powder manufactured in South Africa was recovered and analyzed. Initial identification was attained by utilizing VITEK 2 system, real-time PCR assay and MALDI-TOF MS analysis, following FDA's BAM and manufacturer's procedures. WGS was performed on an Illumina MiSeq system, using a Nextera XT DNA library preparation kit and a 250-bp paired-end read MiSeq Reagent v2 kit (500-cycle), following manufacturer's suggested protocols.

Results: Species identification with high confidence value (>99%) was observed for *C. sakazakii* strain SRL-40 while performing MALD-TOF MS analysis. Genome sequence of this strain was 4,445,879 bp in length, and the draft genome was distributed in 62 contigs. WGS analysis revealed the sequence type as ST64 for the genome of *C. sakazakii* strain SRL-40.

Significance: *Cronobacter sakazakii* ST64 strain has also been recovered from raw ingredients, environment, PIF manufacturing facilities, and various types of food. WGS approaches can be applied for precise strain identification of foodborne *C. sakazakii* isolates.

P2-270 Characterization of the Effect of Probiotics and Essential Oils on Broilers' Resistome

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Introduction: The prevalence of antimicrobial resistance (AMR) in recent years has become a major concern for human and livestock health. Resistome surveillance, or characterizing the collection of all antibiotic resistance genes in a certain environment, allows for improved understanding of AMR selection and spread across multiple sectors within the One Health framework. Specifically in poultry production, potential selection for AMR via diet and/or in-feed antibiotics is a concern. Natural additives such as probiotics and phytotherapeutics are promising replacements for historically used antimicrobial growth promoters to improve broiler chicken health; however, there is a lack of information if they select for AMR.

Purpose: Our objective was to characterize the effects of a probiotic and an essential oils blend on the broiler resistome.

Methods: Cobb 500 1 day-old chicks (N=320) were randomly allocated in 32 cages, with eight replicates of ten broilers per cage per treatment, and were raised until day 21. Treatments consisted of four diets: a basal diet (negative control), a basal diet with Bacitracin Methylene Disalicylate (BMD), a basal

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diet with an essential oil blend, and a basal diet with a probiotic (*Bacillus subtilis*). Stool droppings were collected at three-time points (1, 10, and 21 days) to characterize broilers' resistome. The DNA extracted from these samples was sequenced using shotgun metagenomics on the NovaSeq platform, and statistical analyses were done in RStudio.

Results: Across all samples, 823 unique ARGs were identified. Between treatment groups, no significant differences in Shannon diversity of these genes (Omnibus ANOVA, *p=0.359*) were observed; however, this diversity varied by age (Omnibus ANOVA, *p<0.001)*. In beta diversity, a statistically significant difference was observed across ages (PERMANOVA, *P=0.001*), but not between treatments (PERMANOVA,p= *0.95*).

Significance: While age significantly impacted AMR gene diversity, under our experimental conditions antibiotics or other in-feed additives did not have significant different resistomes.

P2-271 Insights into the Core Soil Microbiomes of Sandy and Clay Soils in Pecan Orchards under Adaptive Multipaddock (AMP) Management Using High-Throughput Amplicon Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Adaptive multipaddock (AMP) grazing is an emerging regenerative agricultural practice which has shown to enhance soil health, improve animal and forage production on the lands, and simultaneously reduce soil greenhouse emissions. Soil microbiomes under AMP grazing could play a significant role in AMP's positive impact in regenerative agriculture management of pecan orchards.

Purpose: The present study aimed to compare the core soil microbiomes of sandy and clay soils in pecan orchards under AMP grazing.

Methods: Six sandy and six clay soil samples were collected from pecan orchards under AMP grazing in Oklahoma. Total soil genomic DNA was extracted using DNeasy PowerSoil Pro Kits. PCR amplification of bacteria-specific hypervariable V4–V5 regions were performed. Positive amplicons were purified using AMpure XP beads and indexed with Nextera XT kit. 600 cycles of paired-end amplicon sequencing were performed in Illumina MiSeq sequencer. Raw reads quality was checked using FastQC. Paired-end reads were merged and filtered using FLASH and Fastp, respectively. Raw tags were compared with the bacteria-specific BugBase databases. For alpha diversity, the Shannon and Simpson diversity metrics were analyzed using the microbiomeSeq R package.

Results: At the bacterial phylum level, Actinomycetota was the most dominant in both sandy (39.24%) and clay (40.11%) soil followed by Pseudomonadota (sandy, 21.12%; clay, 21.26%) and Bacillota (sandy, 16.58%; clay, 10.91%). At the genus level, *Streptomyces* sp. was dominant in sandy soil, whereas *Solirubrobacter* sp. was dominant in clay soil. Alpha diversity analysis revealed that the clay soil samples had the highest bacterial richness and diversity with the Shannon index 6.60 and Simpson index 0.97.

Significance: The high throughput amplicon sequencing could be a useful tool in the study of microbiome communities as influenced by the soil type in pecan orchards under AMP grazing.

P2-272 Genomic Analysis of Drug-Resistant *Escherichia coli* Retrieved from Pre-Harvest and Post-Harvest Fresh Produce

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Introduction: In lebanon, a developing country, the safety of fresh produce faces a myriad of challenges due to the contamination of irrigation water, inadequate agricultural practices, and outdated food safety systems. Despite the importance of fresh produce in the Lebanese diet and recent concerns about foodborne antibiotic resistance in Lebanon, data on the contamination of these foods are limited.

Purpose: We performed in-depth molecular analyses on drug-resistant *Escherichia coli* isolated from pre- and post-harvest fresh produce samples in Lebanon.

Methods: A nationwide surveillance was conducted to evaluate the dissemination of antibiotic resistance (ABR) determinants on pre-harvest (n=60) and post-harvest (n=84) fresh produce across Lebanon. Samples were homogenized (~25 g in 100 mL of buffered peptone water), and an aliquot (100 µL) was spread on RapidE.coli2 Agar; a medium selective for *E. coli* and fecal coliforms in food matrices. Distinctive *E. coli* colonies were selected, and their identity was confirmed by a species-specific PCR. The phenotypic resistance profiles were determined using the disk diffusion assay. Isolates with distinct ABR profiles were selected to analyze their resistome, virulome, pathogenicity, and sequence types (ST) using short-read Illumina sequencing and bioinformatics tools available at the Center for Genomic Epidemiology.

Results: The *E. coli* isolates (n=17) expressed resistance to several important antibiotic classes (cephalosporins, quinolones, aminoglycosides, tetracyclines, phenicol, and folate pathway antagonists), and 64.7% (n=11) were categorized as multidrug-resistant (MDR). Multi-locus analysis (MLST) highlighted the diversity of the detected sequence types. Moreover, the isolates harbored 7-11 acquired antibiotic-resistance genes and various plasmid types. Notably, several isolates harbored resistance genes against commonly used disinfectants and sanitizers (*qacE*, *sitABCD*). All the *E. coli* isolates were classified as potential human pathogens and harbored multiple virulence genes.

Significance: These findings highlight the spread of antibiotic-resistant *E. coli* in pre- and post-harvest produce samples in Lebanon, emphasizing the need to improve agricultural practices.

P2-273 Dissecting Salmonella Serotype Patterns: A Comparative Study Between Poultry Industry Sources and Clinical Samples in the PulseNet Database

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Introduction: Existing methods such as whole genome sequencing (WGS) rely on isolates and a one-sample-one-serotype approach; however, within poultry samples this introduces a risk for misidentifying clinically important serotypes co-present with less pathogenic serotypes.

Purpose: This study sets out to compare serotype data generated from the targeted next-generation sequencing (tNGS) method from poultry sample enrichments with surveillance WGS serotype data from clinical isolates.

Methods: Isolate WGS serotype data from the CDC PulseNet database (n = 174,053, NCBI Bioproject: PRJNA230403), were compared with tNGS data collected from various poultry production facilities across the U.S. (n = 83,899), between January 2020 and December 2023 (4 years). Individual serotype prevalence was compiled over annual quarters for both datasets. The prevalence rate of each serotype was paired across both datasets by year/quarter. A Spearman's correlation analysis was performed on representative serotypes (Kentucky, Typhimurium, and Infantis) to understand the potential similarity or dissimilarity between the two databases and the risk of missing a clinically relevant serotype.

Results: Spearman's correlation analysis on prevalence rates revealed a statistically significant positive correlation for Infantis (p=0.0036), whereas Kentucky and Typhimurium indicated non-significant correlations (p=0.1179; p=0.0781, respectively). Of the 83,899 tNGS samples, 70,134 (83.5%) represented single serotype infections while 13,765 (16.5%) had multiple serotypes (co-presence). Kentucky serotype was identified in 71.79% of these co-presence samples, with Enteritidis/Kentucky and Typhimurium/Kentucky being the most prevalent combinations.

Significance: With 70% of co-presence results including Kentucky and a clinically relevant serotype, this highlights the potential risk/harm of testing for only one serotype through isolate WGS. For applications such as outbreak surveillance, it is critical to consider that multiple serotypes may be present.

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P2-274 Application of Repeated Cycles of Elevated Hydrostatic Pressure Could Improve DNA Extraction, Increasing Sensitivity of Standard and Real-Time PCR Assays for Detection of *Listeria monocytogenes*

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❖ *Developing Scientist Entrant*

Introduction: With advancement and rapid adaption of culture-independent pathogen detection and quantification, innovative DNA extraction procedures could increase sensitivity of standard and real-time PCR (RT PCR) assays, reducing the likelihood of false negatives.
Purpose: Current study utilized a pressure cycle technology to improve DNA extraction procedures and thus increase sensitivity of standard and RT PCR assays for detection of *Listeria monocytogenes* from biotic and abiotic environments.
Methods: Five strains of *L. monocytogenes* and four strains of *Listeria innocua*, *Salmonella* Typhimurium, Shiga toxin-producing *Escherichia coli,* and *Staphylococcus aureus* (as negative controls) were used for standard and RT PCR assays using Bax system Q7 system. The system additionally had an internal positive control for each sample. After purification of the strains, various 10-fold serially diluted samples (McFarland standard of 0.3 to 5.6) were prepared and in addition to standard kit extraction, were subjected to (a) 30 cycles of 240 MPa elevated hydrostatic pressure for 20 seconds per cycle and (b) 100 °C heat treatment to improve the DNA extraction of the samples.
Results: Application of heat and elevated hydrostatic pressure did not interfere with the assays capability of differentiating between the *L. monocytogenes* and negative controls. Similarly, the internal positive controls were not affected by addition of heat and elevated hydrostatic pressure treatments for DNA extraction. While application of heat alone did not meaningfully impact the detection outcome of the assays, utilization of hydrostatic pressure resulted in lower ($p < 0.05$) CT values for samples containing low concentration of *L. monocytogenes.*
Significance: Our results illustrate that application of repeated cycles of elevated hydrostatic pressure could increase the sensitivity of standard and RT PCT assays. Application of elevated hydrostatic pressure could be considered as an alternative to selective enrichment prior to DNA extraction by standard RT PCR kits to improve the detection of pathogens from various biotic and abiotic surfaces.

P2-275 Application of Electronic Nose and Identification of Signature Volatile Compounds for Rapid Detection of Spoilage of Raw Poultry Subjected to Simulated Cold Chain Disruption

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❖ *Developing Scientist Entrant*

Introduction: Cold chain disruptions at various points in the raw poultry supply chain negatively affect the product's shelf life leading to food losses. Volatile compounds emanating during raw poultry storage can be rapid spoilage indicators which can help in product disposition.
Purpose: Research was conducted to characterize and identify signature volatile compounds (VCs) from chicken meat exposed to temperature abuse (TA) to be used as spoilage markers.
Methods: Freshly processed, boneless, skinless chicken breast meat from a commercial poultry processing plant was transported to Auburn University's Department of Poultry Science (4°C). Individual fillets were placed in whirl-pak bags and subjected to TA cycles of 2 hours at 4°C and 2 hours at 25°C. Three regimens were used: (1) 4°C for 24 hours (control), (2) TA for 12 hours, and (3) TA for 24 hours. After TA, the samples were stored at 4°C and evaluated for microbiological spoilage and VCs on days 0, 2, 4, 6, and 8. On each sampling day, three samples were analyzed for aerobic and anaerobic plate counts and Lactic Acid Bacteria (LAB). Additionally, three samples per sampling day were used for volatile compound analysis using an Electronic Nose.
Results: Temperature abuse cycles for 12 and 24 h increased the anaerobic and LAB counts by 1 log, while aerobic counts remained similar to the control samples. Spoilage rate was highest in the 24h TA samples, followed by 12h TA and then by control. E-nose analysis indicated the presence of multiple volatiles, with notable quantitative differences between control and TA samples, particularly on Day 8. Samples subjected to temperature abuse contained volatiles such as acetaldehyde, propanal, trimethylamine, 3-methylfuran, acetoin, and undecane associated with odors such as fatty, ammoniacal, rotten cabbage, rancid and pungent, emerging as indicators of meat spoilage.
Significance: Electronic nose is a highly effective and non-destructive rapid method for spoilage detection of raw poultry.

P2-276 Comparative Genomics for Virulence, Antibiotic Resistance, and Metabolism of Pathogenic *Vibrio parahaemolyticus*

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❖ *Developing Scientist Entrant*

Introduction: Thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH), which are encoded by *tdh* and *trh* genes, are recognized as the major virulence factors in *Vibrio parahaemolyticus*. However, isolates that do not carry these genes can cause illness as well.
Purpose: The objective of this study was to characterize the *V. parahaemolyticus* pangenome and compare the virulence, antibiotic resistance, and metabolism potential of isolates that encode or lack *tdh* and *trh*.
Methods: Genome assemblies for 894 clinical isolates of *V. parahaemolyticus* were retrieved from the National Center for Biotechnology Information database. Quality control, annotation, and pangenome building were performed with CheckM (v1.0.7), Prokka (v1.14.6), and Panaroo (v1.3.4), sequentially. Metabolic features within the pangenome were predicted using eggNOG-Mapper (v2.1.12). The pangenome was translated with Prodigal (v2.6.3) and amino acid sequences were screened for homology to proteins in the Virulence Factor Database and the Comprehensive Antibiotic Resistance Database using BLASTp (v2.14.1). Isolate genomes were divided into four classes depending on the presence/absence of *tdh* and *trh*. Random Forest (RF) analysis was adopted to identify the top-contributing gene signatures for the respective classes.
Results: The analysis yielded 77 *tdh*+, 141 *trh*+, 467 *tdh*+ and *trh*+, and 209 *tdh*- and *trh*- genome assemblies. Regarding virulence, antibiotic resistance, and metabolism, 1,024, 149, and 15,332 genes were identified for each functional category. An accuracy of 0.9026 and a Kappa value of 0.8462 were obtained for the RF model. In differentiating the genomic profiles of the four classes, *vscU2*, *H-NS*, and *TnsA_N* were identified as the most influential genes related to virulence, antibiotic resistance, and metabolism, respectively.
Significance: This study contributes to evaluating the genomic diversity of clinical *V. parahaemolyticus* isolates. We identified key virulence and survival mechanisms with implications for improving ways to manage risks associated with emergent *V. parahaemolyticus*.

P2-277 Proteomic Analysis of Stress-Resistant *Listeria monocytogenes* under Acidic, High Salt Concentration, and Cold Temperature

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❖ *Developing Scientist Entrant*

Introduction: A foodborne pathogen *L. monocytogenes* can survive in stressful environments such as acidic, high salinity, and low temperatures, and can divide two groups stress-resistant and -sensitive *L. monocytogenes* depending on viable cells in the stressful conditions. Although the stress adaptation of *L.*

monocytogenes under those stressful conditions has been steadily studied, proteomic analysis of stress-resistant *L. monocytogenes* was not fully investigated.

Purpose: This research aims to identify the proteome profiles of stress-resistant *L. monocytogenes* using UPLC-MS (Ultra-performance liquid chromatography-mass spectrometer) and to identify biomarkers involved in the resistance mechanism of *L. monocytogenes* in stressful conditions.

Methods: The stress-resistant *L. monocytogenes* strain on three different stressful conditions 1) pH 3 and 1°C; 2) 5% salt and 1°C; and 3) pH 3, 5% salt, and 1°C, and 4) a normal condition (37 °C) was cultured for 12 h and entire proteins were extracted followed by trypsin digestion. Proteomic analysis was conducted using an Orbitrap Fusion Lumos mass spectrometer with a Nano ESI source (Thermo Scientific) coupled with an Acquity M-class UPLC system (Waters). All raw data were analyzed on the Proteome Discoverer 3.0 software and Uniprot database.

Results: A total of 1,319 proteins were identified in all four conditions including ribosomal-related proteins r*npB, rpsL,* and *rpsG*. Interestingly, *L. monocytogenes* virulence protein *InlC* and vancomycin resistance protein *vanR* were identified in three stressful conditions. Proteins *purS, panC*, and *aroB* were only identified in pH 3 and 1 °C, and pH 3, 5% salt, and 1 °C conditions, related to membrane and cytoplasm which are indirectly involved in the virulence pathway and stress response of *L. monocytogenes*.

Significance: Biomarkers related to the stress resistance of *L. monocytogenes* were identified. Understanding the identified proteins associated with the stress response is crucial to understand the mechanism of the stress resistance response of *L. monocytogenes*.

P2-278 Microbial Community Analysis of *Lepidium sativum L.* (cress) and *Eruca vesicaria L.* (rocket), *Brassicaceae* through High Throughput 16S rRNA Amplicon Based Sequencing

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Introduction: Understanding the microbial populations of microgreens is essential for food safety regulations, improving plant health, and human health.

Purpose: This study comprehensively analyses of the microbial communities thriving within *Lepidium sativum* L. (cress) and *Eruca vesicaria* L. (rocket) microgreens belonging to *Brassicaceae* family.

Methods: By using high throughput 16S rRNA amplicon-based sequencing, diverse bacterial communities associated with these microgreens were analyzed in post-harvest period. Through characterizing these communities, crucial insights into the potential impact on the food safety of microgreens are illuminated.

Results: Results of present study indicate distinct microbial compositions within both varieties, elucidating plant-specific microbial colonization patterns. Dominant bacterial phyla, such as Proteobacteria, Actinobacteria, and Firmicutes, were identified, underscoring the prevalent taxa within these niches. This molecular approach unravels the intricate dynamics of microbial communities in *Brassicaceae* plants, contributing to a deeper understanding of plant-microbe interactions and potentially offering insights into harnessing these relationships for agricultural and ecological applications.

Significance: Understanding the role of microbial compositions within these plants is pivotal for assessing and managing potential microbial hazards associated with microgreen production, offering pathways to enhance food safety protocols and ensure the delivery of microbiologically safe and nutritious microgreens to consumers.

P2-279 Emergence of *Salmonella* Newport Strains with Worrisome Multi-drug Resistance Profiles in Pork, Beef, and Surface Waters in Mexico

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Introduction: Multi-drug resistant (MDR) *Salmonella* Newport (SN) strains have recently caused salmonellosis outbreaks in the US and have been linked to beef, soft cheese, and travel to Mexico.

Purpose: To assess the antimicrobial resistance (AMR) profile of SN recently circulating in beef, pork, and surface waters in Mexico, as well as their genetic relatedness to strains involved in clinical cases in the US.

Methods: We conducted antibiotic susceptibility testing (disk diffusion method) and comparative genomic analysis of 88 SN isolates collected from beef, pork, and surface waters across Mexico from 2019 to 2022. These isolates were sequenced using the Illumina MiSeq or NextSeq platforms, and the assembled genomes were analyzed using AMRFinderPlus 3.10 software to predict AMR genotypes. We also assessed the genetic relatedness of these isolates to those involved in clinical cases in the US by consulting the phylogenetic tree on the NCBI Pathogen Detection website.

Results*:* Over 50% of the study SN isolates (45/88) exhibited strong MDR profiles involving 4-8 antibiotic classes. Among these, more than 75% (34/45) also showed resistance to azithromycin, a phenotype that has not been previously reported in environmental isolates from Mexico until recently. The genomes of these MDR isolates harbored multiple AMR genes affecting aminoglycosides (*aadA2*), beta-lactams (*blaCARB, blaSHV-12*), phenicols (*floR*), folate pathway inhibitors (*sul1, dfrA1*), tetracyclines (*tetAD*), macrolides [*mph(A)*], and quinolones (*qnrA1*, *qnrB19*). Except for plasmid-mediated quinolone resistance genes, most AMR genes were of chromosomal origin. Nearly 72% (63/88) of our SN genomes were genetically close (0-10 SNPs distance) to other Newport strains involved in human salmonellosis in the US.

Significance: MDR *Salmonella* Newport exhibiting decreased susceptibility to azithromycin and MDR phenotypes is circulating in beef, pork, and surface waters in Mexico. The widespread dissemination of this potentially deadly pathogen requires urgent interventions to protect public health in Mexico and North America.

P2-280 Systematic Review and Metagenomic Meta-Analysis of Bacterial Communities Harboring *Listeria*

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Introduction: *L. monocytogenes* persistence in food processing environments (FPEs) poses significant food safety challenges, necessitating a deeper understanding of its survival mechanisms within biofilms.

Purpose: We identified all peer-reviewed studies that screened for *Listeria* and performed metagenomics on FPE surface samples and synthesizes their data to identify patterns indicative of *Listeria*'s metabolic advantages within FPE surface microbial communities.

Methods: A total of 464 unique studies were initially screened with 73 qualifying for full-text review. A meta-analysis was performed using R software, incorporating Shannon and inverse Simpson diversity indices. Other data extraction focused on the microbial community composition, specifically the genera correlated with *Listeria* presence through differential abundance analyses. Comparative genomics software tools were employed to elucidate *Listeria*'s potential metabolic interactions within these communities.

Results: Of the 73 studies selected for full-text review, notable exclusions included four culture-dependent metagenomic studies, two exploratory studies with small sample sizes, and nine that were studies of fermentation or spoilage processes. Seven studies were ultimately included in this review. Six of those reported the data required for inclusion in the meta-analysis, the results of which support a nonsignificant relationship between presence of *Listeria* and the alpha diversity of a sample (z, $p = 0.40$). There was significant heterogeny amongst the included studies (τ^2; $p < 0.01$). *Acinetobacter*, *Psychrobacter*, and *Pseudomonas* genera were each identified in multiple studies as significantly higher in abundance in *Listeria*-positive samples. Comparative genomics at the genus-level underscored the importance of cobalamin-dependent genes for *Listeria* growth within biofilms.

Significance: Understanding the metabolic interplay between *Listeria* and other biofilm-forming bacteria offers new perspectives for developing targeted control strategies in FPEs, enhancing food safety and public health.

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P2-281 Bacterial Fermented Oat and Its Benefits in Human Health

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Introduction: *Lactobacillus*-mediated fermentation adds functionality to foods by bioconversion of metabolites which confer multidirectional health benefits.
Purpose: Exploring potential association of an oat postbiotic to provide health benefits via modulating the host immune system and their gut microbiome.
Methods: Two species of *Lactobacillus*, *L. plantarum* 276 and *L. rhamnosus* GG, were used together to ferment 15% oat flour (OF) for 24-h at 37ºC; later pasteurized and freeze-dried to produce oat based postbiotic (OP). OP (1 mg/ml) was used to pretreat a mammalian intestinal epithelial cell line (CaCo2) for 1-h before stimulating them with TNF-α and IL-1β (10 ng/ml each). Immunomodulatory effect of OP was studied by measuring trans-epithelial electrical resistance (TEER), wound healing, and expression of cytokines genes (IL-6, IL-8, and IL-10). Fecal samples from healthy human donors (n=3) were collected to explore bifidogenic, microbiome modulating, and butyrate production ability of OP by RT-qPCR, 16S metagenomic sequencing, and HPLC, respectively.
Results: A significant (p<0.05) restoration of intestinal barrier integrity was observed in presence of OP by measuring TEER. A complete wound closure (p<0.05) was also observed within 24-h. A significant upregulation (p<0.05) of IL-8 was recorded from OP pretreated cells. While expression of other cytokines (IL-6 and IL-10) did not significantly alter in our study. Presence of OP in human fecal media increased Bifidobacteria by 54 times (p<0.05). Overall, 1.35 times more butyrate was detected in the donor's fecal sample who were taking OP for 1 month. Metagenomic study depicted the positive correlation of OP to microbial diversity and richness which was calculated by alpha indices (Shannon, Simpson, Chao1), and total number of species.
Significance: OP has the potential to positively influence human intestinal immunity, microbiome diversity, and butyrate concentration in the gut which in turn may confer health benefits and better protection against intestinal illnesses.

P2-282 Development of Rapid Species-Specific Molecular Methods for Detecting *Cronobacter* Strains from Critical Foods and Environmental Samples

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Undergraduate Student Award Entrant

Introduction: *Cronobacter sakazakii, C. malonaticus* and *C. turicensis,* have been detected in powdered infant formula (PIF) and are known to cause serious infection among infants upon ingestion.
Purpose: There is an urgent need to update the current genus-specific real-time PCR detection method in the FDA Bacterial Analytical Manual (BAM) with species-specific protocols for these three *Cronobacter* species to support laboratory practices for ensuring PIF safety.
Methods: 400+ assemblies of *Cronobacter,* and additional members of *Enterobacteriaceae* were downloaded from NCBI. *C. sakazakii* BAA-894 complete genome (CP000783) was used as input into Primer3 software with a sliding target region and a desired product size range of 150 to 240 bp. Smith-Waterman semi-global alignment was used to select primers with no mismatches to strains of the target species but with maximal mismatches to other species. Primer3 was also used to select flanking primers for unique sequences determined by an in-house *kmer*-binning algorithm. A laboratory workflow consisting of conventional PCR for triaging and real-time PCR (qPCR) for optimizing single and multiplex reactions was developed using a collection of 600+ worldwide PIF, other food samples, and environmental and clinical strains.
Results: A few of the 20 pairs of triaged primers specific to *C. sakazakii (3), C. malonaticus* (2) and *C. turicensis* (2), and a pair co-specific to the first two species were selected after laboratory and bioinformatic testing. Total DNA from mixed cultures of *Cronobacter, E. coli and Salmonella* were used along with 100's of axenic cultures of *Cronobacter* strains to confirm the specificity of the selected primers. Multiplex qPCR protocols with FAM, TAMRA, and Cy5 dyes for genus- and species-specific reactions are being designed and evaluated for sensitivity.
Significance: Accurate and sensitive taxonomic detection of three pathogenic *Cronobacter* species using this rapid and robust molecular method will augment efficient testing of contaminated samples during surveillance and outbreak events.

P2-283 Metagenomic Analysis for Antimicrobial Resistant Organisms Using Nanopore Sequencing Facilitated the Identification and Recovery of a Multidrug Resistant *Raoultella terrigena*

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Developing Scientist Entrant

Introduction: Antibiotic use in aquaculture may lead to the emergence of antimicrobial resistant (AMR) organisms posing a risk to human health.
Purpose: Use of nanopore sequencing to carry out quasi-metagenomic analysis of multiple samples from fish could streamline the process of identifying AMR organisms.
Method: Fish mucous samples (n=12) were each inoculated into a vial of Brain Heart Infusion (BHI) media and incubated at room temperature overnight. Pellets of bacteria were subjected to DNA amplification using the WGA-4 genome amplification kit, followed by use of the 1D ligation sequencing kit to prepare barcoded DNA libraries which were pooled for sequencing on the Mk1C device (Oxford Nanopore). Bioinformatic analyses of the generated sequence data were carried out using the CosmosID Hub (https://www.cosmosid.com) to identify bacterial species and AMR genes. Based on their AMR profiles the remaining inoculated BHI media of bacteria of interest was streaked on BHI agar containing ampicillin (100 µg/ml) and purified colonies of isolates were sequenced individually on the Mk1C sequencer.
Results: Thirty-three bacterial species were detected in the pooled samples and many classes of AMR genes for tetracycline, classes C and D b-lactamases and efflux pumps. Bacterial streaking on BHI agar containing ampicillin led to the recovery of many bacterial isolates including *Raoultella terrigena* which upon nanopore sequencing was found to contain a chromosome of 5.7 Mb and two plasmids of 164 Kb and 82 Kb. The smaller plasmid contained 9 AMR genes: *aadA2* (spectinomycin), *aph(3")lb, aph(6)ld* (aminoglycosides), *sul1, sul2* (sulfonamides), *dfrA12* (trimethoprim), *tet(D)* (tetracycline) and *floR* (florfenicol), *qacE* (chlorhexidine and cetylpyridimium chloride). The chromosome had two AMR genes: *fosA* (fosfomycin) and bla_{TER-2} (ß-lactamase).
Significance: Metagenomics is a powerful technique for analyzing populations or groups of samples towards detecting an organism and its attribute and is poised to play a transformative role in diagnostic microbiology.

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P2-284 Metagenomics for the Identification of Non-culturable Food Spoilage Organisms

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Introduction: It's estimated that 30-40% of the US food supply is lost to food waste with approximately 25% of that due to microbial spoilage. Though quality indicator enumeration is a good trending tool, it cannot reliably predict product spoilage thereby posing a major risk to business. Metagenomics can examine the diversity of the genomes present in a product microbiome including any organisms that may be considered unculturable by traditional means.

Purpose: Discover the organism responsible for blown aseptic product packages observed seasonally during storage at 4°C. Previous attempts with traditional enrichment and plating methods had been unsuccessful in isolating any organism that could be reasonably identified as the cause.

Methods: One intact and two blown aseptic packages of soup, one pre-and one post-chill, were sampled along with chiller water from six production locations. All aliquots were processed using Illlumina DNA Prep for extraction. DNA was quantified with the Invitrogen™ Qubit™4 Fluorometer and libraries prepped with Illumina Nextera® XT v2. Library quality control was evaluated using the Agilent Bioanalyzer 2100 and quantified with Qubit before diluting to 20pM. Sequencing was completed on the Illumina MiSeq™ using the MiSeq™ v3 150-cycle cartridge. The resulting sequence data were analyzed for Taxa via shallow shotgun metagenomics employing the CosmosID® bioinformatics platform.

Results: Analysis of the Taxa models revealed that three extreme psychrophilic yeasts of the genus Mrakia composed 99.94% of the microbial community for the post-chill blown aseptic package and two chiller water samples versus the intact control or pre-chill blown aseptic packages.

Significance: Metagenomics is a relatively inexpensive yet comprehensive way to gather the information necessary to conduct substantive corrective actions for microbial spoilage events even in the case of organisms that have escaped traditional culture techniques.

P2-285 Thermal Inactivation of Salmonella in Plant-based Process Cheese as a Function of pH and Water Activity

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✦ ***Undergraduate Student Award Entrant***

Introduction: While *Salmonella* is known to be a potential hazard in plant-based foods, little is known about how matrix composition affects the thermal inactivation of *Salmonella* in these products.

Purpose: To determine D-values and z-values for *Salmonella* in model plant-based process cheese analogs with varying pH and water activities.

Methods: Four treatments of plant-based process cheese (2x2 block design; pH-4.8 or 5.8; a_w-0.98 or 0.95 were prepared and inoculated with 8-log CFU/g of *Salmonella* (5-strain cocktail). One-gram samples were aliquoted into moisture-impermeable pouches, flattened to 0.5-1.0 mm thickness, vacuum packaged and submerged in pre-heated water baths at one of 5 temperatures (54.4, 57.2, 60.0, 62.8, or 65.6°C). Duplicate samples were removed at specified time intervals and chilled to ≤4°C via ice bath. The log CFU/g of surviving *Salmonella* was enumerated by serial diluting samples in buffered peptone water and plating onto Xylose Lysine Deoxycholate agar overlayed with Tryptic Soy agar. At least three trials were conducted at each temperature. D-values were calculated from the inverse slope of the linear regression of the survival data (log CFU/g) over time and z-values were calculated from the inverse slope of the linear regression of the log of the D-values relative to temperature.

Results: D-values were consistently longer for treatments with pH 5.8 than 4.8 and with a_w 0.95 than 0.98. D-values at the reference temperature of 60°C were 2.34, 13.82, 1.02 and 2.21 minutes for Treatments a_w 0.95/pH 4.8, a_w 0.95/pH 5.8, a_w 0.98/pH 4.8, and a_w 0.98/pH 5.8, respectively and had z-values of 6.73, 5.64, 4.63 and 4.50°C, respectively.

Significance: These results confirm that both matrix pH and aw significantly impact the thermal inactivation rate of *Salmonella* in plant-based process cheese. In comparison to our previous study *Salmonella* was less heat resistant than *Listeria monocytogenes* in the same plant-based process cheese treatments.

P2-286 Microbial and Metagenomic Analysis of Novel Sorghum Kombucha Beverages

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✦ ***Undergraduate Student Award Entrant***

Introduction: Sorghum is known for its high-energy and drought-tolerant properties, traditionally used as livestock feed, and a source of ethanol. In the United States, more than 360 million bushels of sorghum are produced annually. Fermenting sorghum in the form of a tea might create a probiotic beverage that potentially has high concentrations of antioxidants and increased nutritional profiles. Traditionally, kombucha is made from black and/or green tea, sugar, and with a starter culture known as Symbiotic Culture Of Bacteria and Yeast (SCOBY).

Purpose: This study explores the physical and chemical changes, microbial dynamics, and metagenomic profiles of Kombucha during fermentation using three types of whole grain sorghum: white, waxy, and sumac.

Methods: Throughout the 7-day fermentation process of the sorghum extract mixture, pH, °Brix, polyphenol content, lactic acid bacteria, acetic acid bacteria, and yeast and molds counts were observed. Genomic DNA was isolated for shotgun metagenomic analysis sequencing, performed on a NovaSeq 6000 platform (Illumina) to screen for the change in microbiome overtime during fermentation.

Results: Results indicated that the presence of tannins in the sorghum varieties had a noticeable impact on microbial growth, particularly on acetic acid bacteria and lactic acid bacteria. Moreover, significant differences ($P< 0.05$) in polyphenol content were observed among the various treatments, with sumac sorghum Kombucha displaying the highest polyphenol content throughout the fermentation process. The SCOBY consisted mainly of *Brettanomyces* (80%), while *Acetobacteraceae* dominated (95%) the microbial population in the starter liquid. *Brettanomyces* was the most abundant genus in all sorghum Kombucha by the end of the study.

Significance: This study showed the presence of tannins in sorghum can influence the bacterial growth dynamics during fermentation process.

P2-287 The Use of a Cleaner Label Solution to Increase the Shelf-Life of a Plant-Based Meat Alternative Product

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Introduction: Retailers continue to move meat alternatives from the freezer to the refrigerator, which requires cleaner label solutions to achieve a desired shelf-life.

Purpose: Validate a cleaner label solution in a plant-based meat alternative challenged with bacteria isolated from bloated products.

Methods: Phase 1 determined the shelf-life and spoilage of modified-atmosphere packaged CO_2:N_2=20:80 (MAP) patties, MAP-packaged Italian sausage, and vacuum-packaged burger mix stored at 5°C. Products were examined on days 0, 2, 4, 7, 10, and 14 days for pH, organoleptic, and spiral plated in duplicate on nine microbiological media. The plates were incubated, enumerated, and dominant organisms were isolated and characterized. For the challenge study (Phase 2), meat alternative patties formulated with cultured dextrose at either 1.3%, or 1.5%, packaged in either MAP or vacuum, were inoculated, with the Gram-positive gas-producing isolates from Phase 1 at a target of 40 CFU/g, stored at 3.3°C for 32 days, and analyzed twice weekly. At each sample

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point, three experimental units were analyzed for pH, organoleptic, gas composition, and microbial counts on five media. The un-inoculated positive and negative control units were analyzed similarly.

Results: On day 14 of the spoilage determination (Phase 1), total plate and lactic acid bacteria were at 9 log, yeast and mold at 4 log, and *Enterobacteriaceae*, total coliforms, and *E. coli* counts of 2 log or less. The pH dropped from 6.5 to 5.6 over 14 days. The products started to show signs of bloating at 7-10 days. The isolates consisted of 5 yeasts and 21 bacteria, and 3 bacterial isolates were gas-producing, Gram-positive, and catalase and oxidase-negative. Microbial growth was inhibited by the inclusion of the cleaner label solution in the patties challenged with the bacterial isolates, and appropriate shelf life was determined.

Significance: Continue to understand how to extend the shelf-life of meat alternatives.

P2-288 Evaluation of the Risk for *C. botulinum* Outgrowth and Toxin Production in Commercial Plant-Based Meat Alternative Products

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Introduction: Plant-based meat alternative (PBMA) products contain a mixture of plant-derived ingredients, with incoming raw material or additional spices potentially containing spores of *C. botulinum*. Many PBMA products are produced through extrusion, a process in which spores of *C. botulinum* may remain. These products are frequently vacuum-packed or in modified atmosphere packaging and kept in refrigerated temperatures throughout the shelf-life. Potential outgrowth of *C. botulinum* spores is dependent on the storage conditions, with a final heat treatment of the product prior to consumption being a critical step.

Purpose: To evaluate the risk of *C. botulinum* outgrowth and toxin production within a variety of refrigerated, vacuum-sealed plant-based meat alternative products.

Methods: The pH, a_w, and salinity of eleven (11) refrigerated, vacuum-sealed PBMA products (protein source from wheat gluten, soybean, or pea) were surveyed. The endopeptidase-MS assay was verified for botulinum toxin detection in PBMA products using a spike, recovery, and detection method. PBMA products were weighed into whirl-pak bags (25g, in triplicate) and 225mL of Trypticase-Peptone-Glucose-Yeast (TPGY) broth was added. Samples were heat-shocked at 60°C for 15 minutes to inactivate vegetative populations, hand massaged, and anaerobically incubated (37°C) for 7 days in sterile 500mL glass bottles. Samples were evaluated for toxin production using the qualitative endopeptidase-MS assay. Positive controls were included.

Results: The overall matrix characteristics of PBMA products had pH levels of 5.76 – 7.85, a_w of 0.9238 – 0.9972, and salinity of 1.23 – 3.20 ppt. Detection of botulinum toxin in PBMA product was comparable to that of botulinum toxin detection in TPGY. Current results have demonstrated negative toxin production from all PBMA products.

Significance: Findings from this study will aid in determining if the matrix characteristics of PBMA products provide an environment for *C. botulinum* outgrowth and toxin production under refrigerated or temperature abuse conditions.

P3-01 Detection of *Salmonella* from 375 g Dry Kibble Pet Food in 16 Hours Using Hygiena's BAX® System Real-Time PCR Assay

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Introduction: Pet food and treats are well-established vehicles for *Salmonella* contamination. Food safety concerns for salmonellosis exist for both humans and animal companions due to industry use of animal-origin ingredients like human food, as seen by several FDA recalls over the past few years.

Purpose: This study was designed to evaluate the performance of a commercial real-time PCR assay compared to the ISO reference standard for the detection of *Salmonella* from paired 375 g test portions of dry kibble dog food.

Methods: Test portions were inoculated with *Salmonella* at a low level expected to yield fractional positive results and a high level expected to be all positive. Additional samples were left uninoculated as controls. Following inoculation, samples were held at ambient laboratory conditions for 2 weeks. Samples were then homogenized with BPW and incubated at 35° C for 16-24 hours. Paired samples were tested by real-time PCR and confirmed by culture following ISO 6579-1:2017.

Results: Real-time PCR returned positive results for 8/20 low-inoculated samples and 5/5 high-inoculated samples. There was no difference in results when processed with or without a 3-hour BHI regrowth. All real-time PCR results were identical to culture with 100% agreement. Statistical significance using the probability of detection (POD) determined there were no differences between real-time PCR results and reference method results.

Significance: The study results demonstrate that the BAX® System Real-Time PCR assay is sensitive, specific, and accurate for the detection of *Salmonella* in 375 g samples of dry kibble dog food in 16-24 hours.

P3-02 Use of Clean Label Antimicrobial and Natural Flavor to Control Lactic Acid Bacteria and *Salmonella* in Raw Pet Food

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Introduction: High-moisture raw pet food poses spoilage and food safety risks especially *Salmonella*, necessitating the need for an enhanced microbiological safety measure.

Purpose: To assess the efficacy of buffered vinegar and cultured dextrose (Everwild 20L) in combination with smoke system (Cloud S-C100) against specific spoilage (Lactic acid Bacteria) and pathogenic microorganisms (*Salmonella* spp.) isolated from semi-moist pet food.

Methods: Solutions were prepared with 2.5% Everwild 20L and varying concentrations of Cloud S-C100 (0-0.45%) in Brain Heart Infusion (for *Salmonella*) and deMan Rogosa and Sharpe (for LAB). The control without preservative of each media was prepared in the same manner. Samples, adjusted to pH 6.0, were aliquoted into 100-well plates. Wells were inoculated with LAB (*Lactobacillus cellobiosus*, *L. sakei, L. curvatus, Enterococcus faecium, E. faecalis*) or *Salmonella* Typhimurium, *S.* Enteritidis, *S.* Heidelberg, *S.* Mbandaka, and *S.* Agona and incubated at 30°C using Bioscreen-C Pro, 25°C. Growth curves (600 nm) were fitted using modified Gompertz equation to determine the maximum growth rate (μmax; h^{-1}) and lag time (h). Differences among the treatments were determined using one-way ANOVA at $P<0.05$.

Results: Everwild 20L in combination with a minimum concentration of smoke system of 0.3% exhibited bactericidal effect and inhibited both LAB and *Salmonella* serotypes. Without smoke, 2.5% of Everwild 20L alone, significantly ($P<0.05$) reduced growth rate and increased lag time of all the *Salmonella* serotypes ($0.81h^{-1}$;0.32h) compared to the control ($16.6h^{-1}$;0.1h). For LAB, similar inhibition pattern was observed however only *L. curvatus* growth was inhibited by 2.5% of Everwild 20L.

Significance: The blend of buffered vinegar and cultured dextrose and smoke system could potentially function as an effective clean-label antimicrobial and flavor system for raw, high moisture pet food applications. This approach could help pet food manufacturers in humanizing pet food by integrating a clean-label preservation solution.

P3-03 Determination of LOD and RLOD of *Salmonella* in Raw Pet Food Matrices

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Introduction: Each year, there are multiple *Salmonella* outbreaks in raw pet food products. Routine testing of finished products requires accurate detection methods, so it is important to determine and compare the level of detection (LOD) and relative level of detection (RLOD) for faster detection methods to traditional cultural methods.

Purpose: To determine LOD and RLOD of *Salmonella* in three raw pet food matrices using the following methods: Bacteriological Analytical Manual (BAM) culture, Loop-Mediated Isothermal Amplification (LAMP), and PCR methods.

Methods: Raw freeze-dried treats, kibble, and patties were individually inoculated with *Salmonella* at fractional and high inoculation levels. Raw freeze-dried treats and kibble were inoculated at 1, 3, 5, and 7 CFU/25g and raw patties were inoculated at 1, 3, 5, 7 and 10 CFU/25g. Samples were enriched according to the *Salmonella* BAM Section 28 preparation method and assessed using the BAM cultural, LAMP, and PCR methods. Calculation of the POD curves and LOD_{50} values was done using the QuoData web service, μKPI. The BAM cultural method was used as the reference method and the BAM LAMP and PCR were considered alternative methods.

Results: For the BAM cultural method, the LOD_{50} values for freeze-dried treats, kibble, and patties were 0.9, 1.1, and 3.7 CFU/25g, respectively. The RLOD for freeze-dried treats, kibble, and patties using the BAM cultural as reference and, LAMP and PCR methods as alternative methods were 1.1, 0.9 and 0.8, 1.0, 0.8 and 1.1, respectively. The RLODs are close to 1, indicating that LAMP and PCR methods have similar LOD_{50} values as the BAM cultural method.

Significance: *Salmonella* can be detected at low levels in the three matrices tested and the three methods tested had similar LOD50 values. The BAM LAMP and PCR methods can provide results within 24 hours, while the traditional culture method requires 3 days for a negative or presumptive-positive result.

P3-04 Reduction of *Salmonella* spp. in White Grease and Beef Tallow Using Purac® FCC 88

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Introduction: In pet food applications, rendered animal fats are frequently used to coat the exterior of extruded kibble. The rendering process eliminates pathogens, but improper handling of rendered animal fats can re-introduce pathogens such as *Salmonella*.

Objective: To validate *Salmonella* control using lactic acid in white grease and beef tallow.

Methods: For each fat, there were two treatment levels: 0.25% Purac® FCC 88 (lactic acid) and no treatment. White grease was validated at 100°, 110°, 120° and 130°F and beef tallow was validated at 110°, 120° and 130°F. Samples (15 g) were heated in a water bath to the appropriate temperature, inoculated with a *Salmonella* cocktail to reach ca. 6 log CFU/g, and mixed before treatment application. Samples were returned to the water bath and sampled at 0, 15, 30, 60, 90, and 120 min post-treatment application. Sampling was performed by diluting in warmed buffered peptone water containing Tween® 80 and plating on tryptic soy agar plates, which were then incubated at 37°C for 48 h.

Results: In beef tallow, higher temperatures led to a more rapid reduction of *Salmonella* populations in samples treated with lactic acid. When tested at 130°F and 120°F, *Salmonella* was absent in the treated samples after 15 min ($p<0.01$). At 110°F, *Salmonella* was absent in the treated samples after 60 min ($p<0.01$). White grease samples saw a significant *Salmonella* reduction ($p<0.01$) after treatment with lactic acid. Unlike the tallow samples, there was still a small residual *Salmonella* population in later time points in the white grease samples regardless of temperature.

Significance: The application of lactic acid significantly reduced the *Salmonella* population in beef tallow and white grease under conditions utilized by pet food manufacturers.

P3-05 Identification of Animal Tissue from Alfalfa Cubes Linked to a Multi-State *Clostridium botulinum* Outbreak in Horses

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Introduction: In December 2022, FDA's Office of Regulatory Affairs (ORA) received a report regarding nine horses with neurological illness (9/9) and death or euthanasia (8/9) from one farm in Louisiana. The horses shared exposure to a single lot of commercial alfalfa cubes. Visual inspection of the cubes identified embedded animal tissue and fur, and botulism was suspected. In subsequent days, ORA received similar reports from three other states, leading to a multi-state, multi-agency investigation into the source of the outbreak. The Veterinary Laboratory Investigation and Response Network (Vet-LIRN) within the FDA's Center for Veterinary Medicine (CVM) assisted by coordinating identification of the tissue samples found in the implicated product.

Purpose: To confirm botulinum toxin in the product and identify the species of mammalian tissue observed in contaminated alfalfa cubes using a targeted next generation sequencing (NGS) PCR panel.

Methods: ORA's Office of Regulatory Science (ORS) tested samples of the implicated alfalfa cubes for *Clostridium botulinum* toxin using the mouse bioassay. A subsample of the mammalian tissue from the cubes was sent to the Animal Disease Diagnostic Laboratory at Purdue University (PADDL), a Vet-LIRN network lab, for tissue identification using a targeted NGS PCR panel.

Results: *Clostridium botulinum* type C toxin was found in the alfalfa cube samples. Results of targeted NGS of the tissue matched 100% with domestic goat.

Significance: Based on results of this testing, the presence of *Clostridium botulinum* toxin in the alfalfa cubes was likely a result of contamination of the product with decomposed goat tissue containing preformed toxin. This event ultimately affected at least 98 horses and resulted in at least 57 horse deaths. The identification of the mammalian tissue within the alfalfa cubes provided additional information to aid in both root cause analysis and steps to mitigate the risk of similar events in the future.

P3-06 A DNA Purification Method for Continuous Support of the Bovine Spongiform Encephalopathy (BSE) Program

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Introduction: Bovine Spongiform Encephalopathy (BSE) is a fatal neurological disease in cattle that can be transmitted to humans via consumption of contaminated ruminant products. There is currently no treatment or vaccine, so preventing contaminated animal feed from entering food supply is the best means we currently have to reduce the likelihood of BSE transmission. Per 21 CFR 589.2000-2001 and CVM Program 7371.000, the FDA prohibits certain ruminant materials in animal feed and feed ingredients.

Purpose: DNA extraction of animal feeds and feed ingredients followed by a qPCR has been validated to identify prohibited ruminant materials. Due to sample complexity such as different ingredients and processing conditions, qPCR inhibitors may be present in DNA extracts, potentially leading to inconclusive results that need further investigation. Sometimes, repeating PCR or diluting template does not solve the inhibition problem, so a post-extraction purification method was developed.

Methods: Five different DNA purification methods were evaluated, including one precipitation method and four commercial silica gel-based kits. DNA was extracted from archived feed samples, followed by the SybrGreen-based qPCR. Cycle threshold (Ct) and primer melting temperature (Tm) values were reviewed to determine the purification effectiveness.

Results: Our results demonstrated that the precipitation method failed to eliminate qPCR inhibitors from feed DNA, but all four commercially available purification kits effectively served this purpose. Among the four kits, one consistently demonstrated satisfactory efficiency for both positive feeds and negative feeds spiked with reference materials. Furthermore, the removal of qPCR inhibitors by this kit didn't compromise detection sensitivity.

Significance: This study offers a solution to overcome qPCR inhibitors encountered with certain animal feeds. The utilization of a post-extraction purification step shows potential to effectively overcome the technical barrier for continuous method improvement. These results warrant a larger-scale evaluation on method robustness as more archived feed samples become available.

P3-07 Metagenomic Profiling of Animal Food Samples Collected through the Laboratory Flexible Funding Model (LFFM) Cooperative Agreement Program

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Introduction: Although potentially harboring foodborne pathogens, the microbiomes of animal food remain poorly characterized, due in part to low microbial loads and high host tissue content.

Purpose: The study aimed to characterize the microbiomes of diverse animal food samples collected by state laboratories participating in the U.S. Food and Drug Administration's Laboratory Flexible Funding Model (LFFM) Cooperative Agreement Program (CAP).

Methods: From July 2022 to June 2023, four state laboratories (Colorado, Georgia, South Carolina, and Washington) collected 258 samples of dog, cat, rabbit/exotic pet, horse, and swine foods. The samples underwent non-selective overnight enrichment prior to DNA extraction and shotgun metagenomic sequencing on Illumina's NextSeq 1000/2000. Community profiles were established using Kraken2. All samples were cultured for *Salmonella*.

Results: The *Salmonella* isolation rate was low (3/258, 1.2%), which was not correlated with the relative abundances of *Salmonella* reads ($p = 0.47$) in the shotgun metagenome data. Other foodborne pathogens and indicator organisms including *Campylobacter* spp., *Listeria monocytogenes*, *Escherichia coli*, and *Enterococcus* spp. had occasionally high relative abundances, suggesting the presence in those samples. Overnight enrichment prior to metagenomic sequencing resulted in the convergence of microbial communities with a limited number of bacterial genera including *Clostridium*, *Paraclostridium*, *Bacillus*, *Enterococcus*, *Kasakonia*, *Weislessela*, and *Staphylococcus* in most samples. Several correlations existed between animal food commodities and specific microbial taxa, suggesting the establishment of distinct microbial communities in those commodities.

Significance: While shotgun metagenomics is a powerful tool allowing for in-depth characterization of microbial community composition in animal foods, it's utility as a pathogen screening method is unclear. The knowledge gained from this study not only helps advance our understanding of the microbial communities found in these samples but also serve as a guide for future adoption of shotgun metagenomics in animal food microbiome analysis.

P3-08 Identification and Characterization of *Listeria* Species from Raw Pet Food

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Introduction: Raw pet diets can be a transmission vehicle of foodborne pathogens, such as *L. monocytogenes*, that can cause illness to pets and owners.

Purpose: To identify and characterize *Listeria* species from raw canine diets and canine fecal samples.

Methods: Forty-two raw pet diets and 33 canine fecal samples from raw-fed dogs were analyzed for the presence of *Listeria* species by FDA/BAM methods. *L. monocytogenes* and other *Listeria* species were confirmed by polymerase chain reaction (PCR). *L. monocytogenes* isolates were sequenced in Illumina platforms and antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method.

Results: *Listeria* species were identified in 33.3% (14/42) raw pet food samples, from which 8/14 were *L. monocytogenes* and 7/14 *Listeria* spp. Moreover, *L. monocytogenes* was isolated from 3% (1/33) and other *Listeria* species from 6% (2/33) canine fecal samples. Among the non-*monocytogenes Listeria* isolated from food samples 66.7% (4/6) were identified as *L. innocua* and 50% (3/6) as *L. welshimeri* through WGS. From the fecal samples 100% (2/2) were identified as *L. innocua.* The genomic analysis performed to four of the *L. monocytogenes* isolates revealed the presence of the LIPI-1 pathogenicity island and genes encoding resistance to fosfomycin, lincosamides, and streptogramins in 100% of the isolates. Phenotypically, 75% (6/8) of the *L. monocytogenes* isolates showed resistance to sulfamethoxazole/trimethoprim, 66.7% (4/6) *L. innocua* to ampicillin and amoxicillin/clavulanate, and 66.7% (2/3) *L. welshimeri* to tetracycline and sulfamethoxazole/trimethoprim.

Significance: Our findings highlight the public health risks behind the use of raw pet diets, including the transmission of antimicrobial-resistant *Listeria* species.

P3-09 Synthesizing Cleaning and Sanitizing Interventions against *Listeria* spp. Including *L. monocytogenes* in Dairy Processing Facilities: A Systematic Review and Meta-Analysis Approach

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Introduction: *Listeria* poses persistent challenges for processed dairy manufacturers due to its ability to thrive in diverse environments and develop resistance to cleaning measures. Despite the presence of studies examining effective cleaning interventions, the need for a systematic review arises from variations in study size and design, microbial persistence, and resistance to sanitizers.

Purpose: To conduct a systematic review and meta-analysis to evaluate and consolidate information on effective sanitation interventions for *Listeria* spp. across diverse surfaces in a dairy plant scenario.

Methods: Six databases were utilized with terms related to the topic. Terms included *Listeria* spp., Log reductions, intervention strategies (sanitation, antilisterial), and surfaces (stainless steel, glass). Relevance of articles was screened through a two-phase process, i.e., a preliminary screening based on title and abstract and advanced screening based on full texts. Subsequently, data were extracted from identified articles including bibliographical information, study design such as treatment concentration, temperature and time, microbiological methods, and relevant outcomes. A mixed-effect model was applied to describe *Listeria*'s response to sanitizer treatments by considering sanitizer type, sanitizer concentration, biofilm status, surface matrix, treatment time, and temperature.

Results: The literature search yielded 4,677 unique citations, and after applying predetermined inclusion and exclusion criteria, 50 citations were selected for data extraction. Results showed that average *Listeria* inactivation through sanitizers resulted in log reductions ranging from 2.2 to 2.77 log CFU/cm^2. Regarding the physiological state of *Listeria* spp., 70% of the studies investigated *Listeria monocytogenes* in biofilm form, while 22% focused on planktonic stages. The majority of the included studies (73%) assessed log reductions on stainless steel surfaces. Chlorine-based sanitizers were the most frequently studied, followed by quaternary ammonium and peroxyacetic acid sanitizers.

Poster

Significance: The present study synthesized quantitative evidence to increase understanding of the effectiveness of antimicrobial interventions in controlling *Listeria* spp. within dairy processing facilities.

P3-10 Rapid Enumeration of *Lactobacillus* in Dairy Drinks

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Introduction: Dairy drinks fortified with probiotic *Lactobacillus* are very popular in China. Traditional enumeration of *Lactobacillus* follows GB 4789.35-2016, requires 72h of anaerobic incubation on MRS agar plates. *Lactobacillus* is commonly fortified at levels >106 CFU/ml. A laborious dilution is therefore needed. Enumeration of two sequential dilutions is also required. Neogen® Soleris® offers a rapid alternative method to quantitatively detect *Lactobacillus* by adding 1 ml of undiluted sample to the vial DLA-109. CO2 produced by *Lactobacillus* diffuses through a membrane to the agar plug containing a dye indicator. The color change in the dye is read by the instrument. The detection time can be converted to colony counts by a standard curve.

Purpose: This study aim is to compare the performance of the alternative method to GB 4789.35-2016 for *L. paracasei* enumeration in Yili's dairy drinks.

Methods: Dairy drinks from Yili were diluted by boiled counterparts to make serial dilutions. For each dilution level, 1 mL of sample was added to a DLA-109 vial for alternative method detection. The MRS agar method was performed following GB 4789.35-2016. A standard curve was generated by plotting MRS colony counts against alternative method detection time. 20 samples were then tested by both methods for comparison.

Results: The standard curve was y = -0.2024x + 10.36, where y is the log CFU and x is the detection time. The correlation coefficient is -0.9996. For the 20 samples tested by both methods, log CFU differences were between -0.19 and 0.23, indicating that the alternative method is very comparable to the MRS agar method.

Significance: The rapid alternative method demonstrated accurate results in less than 16 hours and was a comparable method to the MRS agar method for enumeration of *Lactobacillus* in dairy drinks. The reduction in incubation time can save food factories 2 days of additional incubation time and help free up storage space.

P3-11 Fermentate, Vinegar and Plant Extract Based Clean Label Solutions to Replace Potassium Sorbate in Salad Dressings and Sauces

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Introduction: Microbial spoilage in sauces and salad dressings, by lactic acid bacteria and yeast, remains a challenge. Increasing consumer desire for clean label products underscores the need for incorporating natural antimicrobials in sauces and dressings.

Purpose: Three clean-label preservation system formulations were tested for their inhibitory effects in yeast and lactobacilli in a salad-dressing model over 9-months of storage.

Methods: Three clean-label antimicrobial formulations, i.e., plant extract A + 0.7% vinegar (T1), plant extract B + 0.7% vinegar (T2), and a combination of cultured dextrose and vinegar (T3), were incorporated into a salad dressing model. 0.1% potassium sorbate was used as a conventional control (T4). Each treatment was separately inoculated with a lactobacillus (LAB) cocktail (*Lactobacillus plantarum*, *L. buchneri, L. brevis, L. fermentum and L. fructivorans*) and a yeast cocktail (*Candida lusitaniae, Debaryomyces hansenii, Saccharomyces cerevisiae, Zygosaccharomyces bailii,* and *Pichia membranaefaciens*), with a target inoculum level of 3 log CFU/g. A portion of each treatment was left uninoculated as the negative control. Duplicate samples of each treatment were analyzed weekly for 4 weeks, followed by monthly assessments for up to 9 months, to monitor the counts of LAB and yeasts. Data was analyzed using one-way ANOVA at $p<0.05$.

Results: The LAB and yeast count in the negative control remained below detection limit throughout the study (<1 log CFU/g). For control samples (inoculated with no treatments), the LAB and yeast counts exceeded 6 log CFU/g within 21 days. For all the formulations, the detectable levels of LAB went <1 log CFU/g within 7 days, whereas for the yeast counts, the detectable levels went <1 log CFU/g within 14 days for T1, T2, and T3, and within 7 days for T4, exhibiting bactericidal effects of the antimicrobial formulations. There were no significant differences ($p<0.05$) among the treatments.

Significance: Natural clean label formulations are effective in inhibiting LAB and yeast growth in salad dressings.

P3-12 Analysis of Method Performance for Quantitative Assessment of *Listeria monocytogenes* in Queso Fresco Cheese

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Introduction: Quantitative assessment of *Listeria monocytogenes* is important to analyze its level in a food product. FDA BAM method is commonly used for detecting and enumerating L. monocytogenes in cheese; however, performance characteristics of the enumeration method is not widely available in the literature.

Purpose: In 2023, the Moffett Proficiency Testing Laboratory (MPTL) organized a PT for Food Emergency Response Network laboratories to determine the ability of participants to enumerate *L. monocytogenes* in queso fresco cheese and evaluate the performance of the enumeration method.

Methods: MPTL conducted preliminary studies and the pre- and post-shipment assessment to evaluate stability and homogeneity of the inoculated samples according to ISO/IEC 17043:2010 and ISO 13528:2022 standards. Two 50 g test samples were prepared for each participating laboratory with 10 CFU/g of *L. monocytogenes*. MPTL also participated in the PT along with twenty participant laboratories. In all cases, samples were analyzed using BAM method.

Results: Mean recovery rate in preliminary studies was 114%, and the confidence interval included the value of 100% indicating no evidence of systematic deviations. Samples tested at pre- and post- shipment stages were within tolerance range of 2.10 CFU/g and 24.00 CFU/g. MPN data obtained by the participants had discrepancies; therefore, the tube readings were used to recalculate MPN values. Based on the Q/Hampel method (ISO 13528:2022), the repeatability standard deviation was 0.308 log MPN and the reproducibility standard deviation was 0.444 log MPN. The resulting laboratory standard deviation was 0.320 log MPN indicating the extent of systematic deviations among participants similar to the intrinsic random variation of the MPN procedure. Overall, the procedure had an acceptable performance, and the participants were able to perform MPN method for the given inoculation level.

Significance: Participation in such PTs can help generate quantitative data to determine accuracy of a specific method, laboratory and analysts' performance.

P3-13 The Evaluation of Soleris® Rapid Method as an Alternative GB Method for Commercial Sterility of Dairy Products

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Introduction: Commercial sterility methods play a vital role in the dairy industry. However, the traditional method, GB 4789.26-2023 for commercial sterility test, needs a 7–10-day incubation followed by multiple steps. Market competition and plant warehouse management induced the research of a shorter time-to-result test method for ultra-high temperature (UHT) dairy products.

Purpose: To develop shorter incubation and detection times for UHT dairy products compared with traditional methods.

Method: We collected 8 types of modified milk and 5 low pH dairy drinks. All 15 matrices were artificially contaminated with *Bacillus subtilis ATCC 6633, Geobacillus stearothermophilus ATCC 7953, E. coli CMCC(B)44102, Saccharomyces cerevisiae ATCC 9763,* respectively. Each organism was spiked at low (10^0-10^1 CFU/g) levels. The 8 modified milks were incubated at 36°C for 32 hours, 34 hours, 36 hours, and 38 hours, and the 5 low pH diary drinks were incubated at 36°C for 42 hours and 48 hours. The statement of samples was identified with both reference method (plate streaking/count at 36°C for 2 days) and alternative method (Neogen® Soleris® NF-105 vials at 36 °C for 24 hours).

Results: A paired qualitative analysis method was performed. For all the incubation time sets, the dPOD values were between the LCL and UCL. The relative trueness analysis of modified milk showed the bias are 89.1%, 92.2%, 98.4% and 90.1% for 32 hours to 38 hours, respectively. The low pH dairy drinks inhibited all the bacteria except *S. cerevisiae*, which presents 100% and 97.9% relative trueness for 42 hours and 48 hours.

Significance: The alternative commercial sterility rapid method for UHT products demonstrated the possibility of no more than 36-hour incubation with 24-hour test method. This method presented not only accuracy and reliability, but also timeliness and efficiency.

P3-14 Identifying What Drives Small and Medium Dairy Plants to Invest in *Listeria* Environmental Monitoring Programs

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Introduction: Small and medium sized dairy production facilities (SMDPFs) may face challenges (e.g., due to limited financial resources) when implementing their food safety programs, including *Listeria* Environmental Monitoring Programs (EMPs). EMPs may receive less investment due to the limited resources and possible underappreciation of food safety programs by SMDPFs.

Purpose: Survey nine SMDPFs to better understand what drives their *Listeria* EMP investment decision making.

Methods: All SMDPFs implemented *Listeria E*MPs in their facilities over one-year with the help of a team of five trained Extension Associates and Graduate Students. At the end of the study period, the team utilized a pre-determined rubric to assess EMP Commitment Scores for each facility using the Likert Scale. Additionally, the nine SMDPFs were evaluated using a questionnaire distributed via email assessing their self-reported EMP associated costs (e.g. cost of corrective actions, total cost of EMP, estimated total value of finished product (ETVFP).

Results: Facilities were divided into three groups based on their ETVFP. Group 1 (n=4) which had an ETVFP of <$50,000, invested a mean of $7,755/year into their *Listeria* EMP. Group 2 (n=4) had an ETVP between $50,000 and $200,000 and invested a mean value of $18,271. Finally, Group 3 (n=1) had an ETVFP >$2,000,000, and invested $5,000 into their *Listeria* EMP. There was no correlation between investment into EMP and overall *Listeria* prevalence, facility size, ETVFP, and EMP commitment score (p=0.502).

Significance: EMP investment drivers could not be determined in this study indicating that investment into EMP is based off other potentially, irrational factors. A deeper understanding of EMP investment drivers is imperative so that better and more targeted strategies surrounding food safety support can be developed.

P3-15 Effect of the Bioprotective Properties of Lactic Acid Bacteria Strains in Aerobic Storage of Feta Cheese Inoculated with *Listeria monocytogenes*

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Introduction: Lately, the addition of lactic acid bacteria (LAB) strains to cheeses is gaining more attention, since they can impact their nutritional, technological and sensory properties, as well as improve the safety of the product.

Purpose: This work studied the effect of *Lactiplantibacillus pentosus* FMCC-B281 and *Lactiplantibacillus plantarum* FMCC-B-282 free cells and supernatants on the fate of *Listeria monocytogenes* on aerobic stored Feta-cheese slices.

Methods: Slices of Feta cheese were contaminated with 4 log CFU/g of *L. monocytogenes* and then the cheese was sprayed with i) free cells (F, ~5 log CFU/g), ii) supernatant (S) and control (C, UHT milk). Then, samples were wrapped with domestic membrane and stored at 4 and 10°C. During storage, microbiological and pH were monitored, while sensory assessment was conducted.

Results: Results showed that the initial microbial population of Feta was ca. 7.6 log CFU/g and consisted of LAB (>7 log CFU/g) and Yeast-Molds in lower levels (ca 3 log CFU/g), while no *Enterobacteriaceae* were detected. Initial pH was 4.2. During cold storage, pathogen population increased by 4 logs, while Yeast-Molds population was found to be 6.4 log CFU/g and pH was 5.4 at control, whereas at S and F-samples pathogen growth was postponed and reached slightly lower levels by the end of shelf-life. At 10°C, population of Yeast-Molds and *Enterobacteriaceae* achieved high populations (>7 log CFU/g) by the end of storage. Shelf-life was shorter at control samples, in contrast to S and F-samples where their shelf-life was elongated by 8 and 12 days, respectively. Sensory assessment revealed that S-samples were better compared to control and F-samples.

Significance: The applied bioprotective strains can elongate Feta shelf-life and provide a mild antimicrobial action against foodborne pathogens and spoilage microbiota.

P3-16 Prevalence of Listeria spp. in Traditional Serbian Dairy Products

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Introduction: Serbia produces an average of 1.5 billion liters of raw milk annually, and almost half of all milk is processed by small scale dairy producers (SSDPs). The main dairy products produced by SSDPs include white brined and soft cheeses, as well as kajmak, a specialty dairy product originating from Serbia. The nature of these products and their processing steps makes them especially vulnerable to *Listeria monocytogenes* contamination and growth. Given the high mortality rates associated with *L. monocytogenes*, it is imperative to understand the prevalence of this pathogen in traditional Serbian dairy products.

Purpose: To investigate the prevalence of *Listeria* spp. and *L. monocytogenes* in traditional dairy products from SSDPs in Serbia.

Methods: A total of 302 samples of dairy products produced by SSDPs were collected from open markets and retail establishments in Serbia, including raw milk cheeses (n=79), pasteurized milk cheeses (n=111) and kajmak (n=112). *Listeria* spp. were isolated using ISO 11290-1 method and confirmed using API *Listeria* test (bioMérieux, France). Species identification from single colonies was performed by MALDI-TOF MS (VITEK, bioMérieux, France).

Results: Overall, low prevalence of *L. monocytogenes* was observed, with 1.3% (n=1) of raw milk cheese and 4.5% (n=4) of kajmak samples positive. Other species recovered included *L. innocua* in pasteurized milk cheese (n=1; 0.90%) and kajmak (n=4; 3.6%), and *L. ivanovii* in one kajmak sample (0.90%).

Significance: Our data show that traditional Serbian dairy products have overall low prevalence of *L. monocytogenes.* Kajmak products were the most prone to *Listeria* spp. contamination. As kajmak is produced from pasteurized milk, the contamination is most likely from handling during processing and distribution. To prevent contamination in facilities handling traditional dairy products it is therefore crucial to ensure high raw milk quality, an effective pasteurization process, hygienic production, and storage conditions, and appropriate cleaning and sanitation procedures.

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P3-17 Evaluation of the TEMPO® AC Method for the Enumeration of Thermophilic Aerobic Bacteria in Dairy Raw Ingredients

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Introduction: Enumerating thermophilic bacteria in dairy powder and infant formula is an important part of quality programs for these manufacturers as it could potentially impact pricing and consumers. A rapid automated system for the enumeration of quality indicators was investigated to determine the efficacy of a newly developed thermophilic protocol**.** The following raw ingredients were analyzed: raw milk, pasteurized milk, whey and whey protein concentrate. The automated method was compared to classical plate count agar (PCA) enumeration.

Methods: One hundred twenty-five total samples of dairy raw ingredients were evaluated for this study in a paired test portion comparison investigation. All test portion comparisons were naturally contaminated. AC cards were incubated at 55°C to facilitate the accurate enumeration of thermophilic bacteria.

Results: When comparing the automated method to traditional thermophilic aerobic plate counts using PCA, 121 out of the total of 125 comparisons agreed within 1 log for an overall rate of 97%.

Significance: The AC method incubated at 55oC for the enumeration of thermophilic aerobic bacteria is a rapid, easy-to-use automated alternative to manual and subjective plating procedures for the enumeration of quality indicator organisms. These data demonstrate that the AC is a suitable method for the enumeration of thermophilic aerobic bacteria in food products. This extends the applicability of the TEMPO AC disposable to both mesophilic and thermophilic aerobic bacteria enumeration.

P3-18 Factors Influencing the Level of Detection of Listeria monocytogenes in Ice Cream

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Introduction: *Listeria monocytogenes* has caused several multi-state listeriosis outbreaks of ice cream in recent years. Factors influencing the detection of *L. monocytogenes* in ice cream need to be studied.

Purpose*:* Evaluate factors influencing the sensitivity of the FDA Bacteriological and Analytical Method (BAM) method for detecting *L. monocytogenes* in ice cream.

Methods: Naturally contaminated ice cream from the 2015 *L. monocytogenes* outbreak (stored at -20°C) was used to prepare the test portions in this study. A factorial study as described in the ISO 16140-4 standard was used to evaluate the detection of *L. monocytogenes* in ice cream with the FDA BAM method. The following factors were studied: test portion size (10 g, 25 g), types of ice cream, fast or slow thawing, with or without refreezing, and different chromogenic plating media (MOX, RLM, R&F). Sensitivity was measured by Level of Detection (LOD) and Relative LOD (RLOD) and compared across different factorial levels.

Results: The naturally contaminated ice cream contained 2.15 MPN/g *L. monocytogenes*, consistent with that (0.15-7.1 MNP/g) reported in 2016. LOD_{50} values for the test portion size 10 g and 25 g were in the range of 0.6 to 0.7 CFU/ test portion. The larger test portion size (25 g), however detected 2.5 times lower levels of *L. monocytogenes* than the 10 g portion size, indicating higher sensitivity. Types of ice cream, thawing, and refreezing conditions in the combined analysis (when 10g and 25g test portion sizes considered together) did not affect LOD_{50} and RLOD. The three different media (MOX, RLM, R&F) indicated no significant effect on the sensitivity.

Significance: ISO 16140 factorial design approach proved to be a valuable technique for studying factors affecting the sensitivity (LOD50 and RLOD) of microbial methods.

P3-19 Enhancing Microbial Safety and Quality of Milk with Ultrasonication: Kinetics Modeling of Pathogenic Bacteria and Milk Characteristics

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Introduction: Traditionally, thermal treatments are employed for the microbiological safety of milk, which further extends the shelf-life. However, ultrasonication continues to gain interest as a novel non-thermal technology to guard against possible foodborne pathogens, while also minimizing adverse effects on product quality.

Purpose: To investigate the inactivation kinetics of *Staphylococcus aureus* NCDC109, *Escherichia coli* EMC17 and *Salmonella* Typhimurium SMC25 in ultrasonicated raw whole milk at different frequencies, and to determine the impact on milk quality.

Methods: Inoculated milk samples (50 mL) were sonicated at different ultrasonic amplitudes (40, 50, 60, and 70%) by submerging the probe within ~2 cm of the milk samples and treating for 0, 2, 5, 10, 15, 20, 25, or 30 min. Ice jackets were placed around the beaker containing the milk during ultrasonication to mitigate the thermal impact of the ultrasonic waves. Physicochemical characteristics (particle size and zeta potential, pH and temperature, viscosity) were examined. To generate survival curves, kinetic data were fitted to two inactivation models, linear and Weibull model, using the USDA Integrated Pathogen Modeling Program (IPMP) 2013 software.

Results: The Weibull model accurately predicted D-values for all strains and treatment amplitudes, with Af values ranging from 1.02 to 1.01 and Bf values of either 1.00 or 1.01. The time required for a 5-log reduction of *E. coli* EMC17 significantly decreased from 46.05 min for 40% amplitude to 20.01 min for 70% amplitude. Similar results were obtained with the other two pathogens. Furthermore, ultrasonication did not result in significant changes in the physiochemical characteristics of milk, regardless of the amplitude or time.

Significance: Results from this study serve as a platform for the development of ultrasonicated milk processing protocols with comparable outcomes to current pasteurization technologies.

P3-20 Assessing the Efficacy of a Commercial Probiotic in Preventing Colonization of *Listeria monocytogenes* on Wooden Cheese Aging Boards

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Introduction: Wood is contentious as a cheese aging surface because of its potential to harbor pathogenic microorganisms and the difficulty in effectively sanitizing it.

Purpose: This study assessed the utility of pre-inoculating wooden cheese aging boards with probiotic bacteria for preventing the colonization of *Listeria monocytogenes* and how boiling as a sanitation method affects *Listeria monocytogenes* colonization of the boards.

Methods: On two separate occasions, boards (n=40) were divided into four groups (10 boards/group): The commercial probiotic, SuperSile Dry Hay inoculant, made into yogurt, the probiotic diluted in autoclaved store-bought yogurt, the probiotic diluted in sterile phosphate-buffered saline (PBS), and sterile PBS alone. Within each group, 5 boards had been previously boiled 3 times, while 5 had never been boiled. Each board was inoculated with 3-4 log CFU/ml of *L. monocytogenes* and subsequently incubated for three weeks at 11°C in wine coolers. The boards were aseptically chiseled, and wood shards enriched

and enumerated for *L. monocytogenes* with most probable number (MPN) according to methods described in the FDA BAM manual. The log reduction in *L. monocytogenes* colonization was determined by comparing the Log MPN for each trial condition to the initial inoculum applied to the boards. The resulting means were compared using one-way ANOVA to determine statistical significance.

Results: Boards treated with the probiotic-based yogurt and the probiotic in PBS had the highest log reduction in *L. monocytogenes* colonization, 2.07±0.61 MPN/g and 1.85±0.83 MPN/g respectively. These were significantly higher (p=0.008) than the log reduction in *L. monocytogenes* colonization for boards treated with SuperSile in sterile yogurt (0.10±0.58 MPN/g), which did not significantly differ from boards treated with just PBS. Boiling history did not significantly affect *L. monocytogenes* colonization of the boards.

Significance: Probiotics have the potential to mitigate *L. monocytogenes* colonization on cheese ageing boards but may not offer complete protection especially in significant contamination scenarios.

P3-21 Identification and Evaluation of Bioactive Fractions Derived from Bioconverted Milk Having Anti-Inflammatory Effect in RAW264.7 Macrophages

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Introduction: In recent years, considerable research has been dedicated to isolation and validation of the bioactive compounds present in natural products, with a focus on their efficacy in ameliorating diseases.

Purpose: This study aimed to investigate which bioactive fractions are major determinants of the anti-inflammation potential of bioconverted Artemisia herba-alba extract-added milk produced by *Lactiplantibacillus plantarum* SMFM2016-RK (BM2) on RAW264.7 cells.

Methods: A total of six solvents (methanol, hexane, ethyl acetate, butanol, ethanol, and water) were used to fractionate the bioactive compounds from BM2. All fractions were evaporated in a rotary evaporator, and suspended in distilled water. The cytotoxicity of each fraction in RAW264.7 cells was assessed by means of the the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) colorimetric assay, and the concentration that exhibited no cell death was chosen for further analysis. Porphyromonas gingivalis LPS-induced RAW264.7 cells were treated with each fraction, and the nitric oxide (NO) expression level in the cells was measured. Polyphenolic compounds, lipids, flavonoids, saponins, non-polar substances, and amino acids from BM2 were fractionated by methanol, hexane, ethyl acetate, butanol, ethanol, and water solvents, respectively.

Results: The optimal concentrations of six fractions, which exhibited no significant difference in cell viability compared to the negative control, were found to be methanol 1:1000, hexane 1:10, ethyl acetate 1:250, butanol 1:100, ethanol 1:100, and water 1:100, respectively. All of the bioactive fractions significantly lowered the expression of NO in LPS-induced RAW264.7 cells (16.0 μM), and furthermore, the NO expression levels in cells treated with methanol (0.6 μM), hexane (2.5 μM), ethyl acetate (1.5 μM), butanol (0.5 μM), and ethanol (0.9 μM) fractions were not significantly different from those in normal cells (0.9 μM).

Significance: In this study, among the bioactive compounds fractionated from BM2, methanol, hexane, ethyl acetate, butanol, and ethanol fractions displayed anti-inflammatory properties based on the NO expression level results.

P3-22 Anti-Inflammatory Effects of Amino Acids from Milk and Artemisia herba-alba by Bioconversion on YD-38 Human Oral Squamous Carcinoma Cells

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Introduction: Bioactive compounds produced in fermented milk, such as amino acids and peptides have important physiological and biochemical functions that enhance the immune system. Previous studies have shown that Artemisia herba-alba extract-added milk bioconverted by Lactiplantibacillus plantarum is effective against periodontal inflammation.

Purpose: The purpose of this study was to analyze the anti-inflammatory effects of amino acids derived from *A. herba-alba* extract-added milk bioconverted by *L. plantarum* SMFM2016-RK.

Methods: Two types of bioconverted milk were prepared by adding *L. plantarum* SMFM2016-RK only (BM1) and *L. plantarum* SMFM2016-RK and *A. herba-alba* extract (BM2), and the amino acids derived from bioconverted milk were quantified by high performance liquid chromatography (HPLC) with fluorescence detector and UV detector. Amino acids with significantly higher amounts in BM2 compared to those in BM1 were selected as bioactive compounds. YD-38 cells were treated with 0.05 mM hydrogen peroxide to induce oxidative stress, and simultaneously the selected amino acids were added to the cells. The cells were incubated at 37°C and 5% CO_2 for 3h. The mRNA of cells was extracted, then their cDNA was synthesized from the mRNA to analyze the expression of inflammation markers (*TNF-α, IL-1β, IL-6, iNOS, COX-2*) using real-time PCR.

Results: HPLC analysis revealed that proline and asparagine were produced in BM2 significantly more than in BM1. The asparagine amount in BM2 (786.72 mg/L) showed 16-fold higher than that in BM1 (48.04 mg/L). Also, the proline amount in BM2 (532.34 mg/L) showed 10-fold higher than that in BM1 (57.83 mg/L). RT-PCR analysis showed that the expression of *TNF-α*, *iNOS*, and *COX-2* in YD-38 cells significantly decreased with the addition of asparagine(p<0.05). Also, the expression of *TNF-α, IL-1β*, and *COX-2* in the cells tended to decrease upon adding proline to the cells, but with no significant difference.

Significance: These results indicate that proline and asparagine derived from A. herba-alba extract-added milk bioconverted by L. plantarum could alleviate periodontal inflammation.

P3-23 Population Dynamics and Bidirectional Transfer of *Listeria monocytogenes* and Shiga Toxin-Producing *Escherichia coli* During Cheese Production in Wooden Vats

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Introduction: The safety of using wooden vats for cheese production remains controversial as the porous structure of wood may harbor pathogens and protect them from cleaning and sanitation processes.

Purpose: The present study determined the survival of *Listeria monocytogenes* and Shiga toxin-producing *Escherichia coli* (STEC) during the production of St. Nectaire-type cheese in wooden vats as well as their ability to transfer to the wood and then to subsequent batches in the absence of formal cleaning.

Methods: Four batches of St. Nectaire-type cheese were produced in triplicate in seasoned 1.5L wooden vats. The first and third batches were made with raw milk inoculated with L. monocytogenes at ~2 log CFU/mL and STEC at ~1 log CFU/mL, respectively. The second and fourth batches were made with uninoculated raw milk. Swab samples from vat walls and floors were collected after cheese production. Pathogen counts in milk, cheese, and swabs were determined by plating on CHROMagar and analyzed by repeated measures ANOVA with Tukey HSD. Samples with counts below the limits of enumeration (1- 5 CFU/mL or g; 0.22 - 1.43 CFU/cm^2) were enriched using standard methods.

Results: Counts of both pathogens increased in fresh curd (0.88-1.79 CFU/g) and again in cheese after salting and overnight pressing (~0.5 CFU/g) (p<0.04). *L. monocytogenes* was present on the walls of all three replicate vats after cheese production using inoculated milk but was absent in all cheeses subsequently produced from uninoculated milk. STEC was not detected on vat surfaces after cheese production with inoculated milk or any dairy samples

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when used subsequently to produce cheese from uninoculated milk.

Significance: The limited ability of pathogens to colonize the wooden vats and contaminate subsequent batches, despite the ability to grow during cheese production, suggest that the risks of using wooden vats to produce cheese is low if the milk is of high microbiological quality.

P3-24 Characterization of Mammary Pathogenic *Escherichia coli* Isolates from Bovine Mastitis in South Korea

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Introduction: Mammary pathogenic *Escherichia coli* (MPEC) is a major etiological pathogen of bovine mastitis that causes huge economic losses in dairy industry worldwide. However, our understanding of the clonal distribution and common virulence traits of MPEC isolates in South Korea is limited.

Purpose: In this study, we investigated the clonality, virulence genes and antibiogram of MPEC isolates from milk of dairy cows with mastitis collected from five different provinces in South Korea.

Methods: A total of 92 MEPC isolates belonging to phylogroup A (n=24) or B1 (n=68) were examined to determine (i) the clonal lineages of the isolates by discriminating sequence type (ST) and O-antigen type, (ii) profiles of virulence genes, and (iii) antimicrobial resistance (AMR) patterns.

Results: The results highlight the clonal diversity of MPEC isolates and suggest that ST10, ST58, and ST906 may be more successful than other STs at causing mastitis. Interestingly, the *fimH*, *ecpA*, and *traT* were present in most of the isolates, while other virulence genes were detected only sporadically. As compared to a previous report, the susceptibility to antimicrobials still remains high among the MPEC isolates, but the resistance rate to ceftiofur, a third-generation cephalosporin, was increased. Notably, we identified the possible endemic clones of the multidrug-resistant (MDR) MPEC isolates from different dairy farms: ST10-O9 and ST58-O8.

Significance: This study is the first to provide insights to the clonal diversity and major virulence factors of MPEC isolates in South Korea, as well as reveal the spread of MDR clones throughout dairy farms that can be a serious threat to public health.

P3-25 Review of Historical *Listeria monocytogenes* Outbreaks Linked to Soft Cheeses between 2011–2023, Existing Challenges, and Potential Prevention Efforts

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Introduction: From 2011 to 2023, there have been 14 multi-state outbreaks of *L. monocytogenes* (a foodborne pathogen that primarily affects pregnant women, infants, the elderly, and immunocompromised) associated with soft cheeses made from pasteurized and unpasteurized milk. The recurrence of these outbreaks associated with pasteurized, soft cheeses suggests deficiencies with food safety practices.

Purpose: To analyze historical outbreak data from *L. monocytogenes* outbreaks associated with pasteurized and unpasteurized soft cheeses and identify significant trends and prevention strategies.

Method: Outbreak data from the 14 *L. monocytogenes* outbreaks associated with soft cheeses was reviewed and compared. Significant similarities and differences within the data were further analyzed, such as case-patient exposures, product/environmental samples, and inspectional findings. Conclusions were made from the analysis to identify additional prevention efforts.

Results: Eleven of 14 outbreaks were linked, or suspectedly linked, to soft cheeses made from pasteurized milk. During all 14 outbreaks, 141/150 cases were hospitalized. Fetal loss occurred among 10/35 hospitalized, pregnant women during 8/14 outbreaks. Environmental and/or cheese samples collected at manufacturers during 10/14 outbreaks, and product samples collected at retailers during 7/14 outbreaks, were analyzed and found positive for *Listeria spp*. During all 14 outbreaks, inspectional deficiencies were related to equipment issues, sanitary operations, plant and grounds construction/design, personnel practices, processes and controls, food safety plan requirements, and hazard analysis. Identified prevention efforts are based in continued enforcement of the Current Good Manufacturing Practice, Hazard Analysis, and Risk-Based Preventive Controls for Human Food Rule (PCHF rule) and education and outreach.

Significance: Inadequate sanitation procedures at manufacturers are potential contributing factors to the contamination of pasteurized soft cheese by *L. monocytogenes*. The findings from the data analysis highlight the need for continued enforcement of the PCHF rule and the importance of educational outreach to consumers (particularly pregnant women), industry, and retailers regarding this pathogen and the rule.

P3-26 *Salmonella* Reduces the Bacterial Diversity in Milk and Requires Fur-mediated Iron Metabolism for Milk Colonization

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❖ ***Developing Scientist Entrant***

Introduction: Milk, a highly nutritious food matrix, is rich in antimicrobial factors such as lactoferrin and a complex microbiota important in maintaining food quality and safety.

Purpose: Failure to maintain the appropriate temperature conditions during transit and storage can result in the contamination of milk samples with foodborne pathogens, such as *Salmonella*. Citing well-characterized examples from the gut, we hypothesized that *Salmonella* must overcome the competition from the milk microbiota before colonization.

Methods: To test this, we explored how the resident milk microbiota is altered during temperature abuse and pathogen contamination using PacBio-based whole 16S rRNA sequencing. A total of 60 samples were included in this investigation.

Results: Our results show that, in both experimental conditions, bacterial diversity was significantly decreased. The populations of *Thermus* and *Streptococcus*, prevalent at 0 hours, were greatly diminished during 12 incubations at 37°C, representing temperature abuse. Similarly, the presence of *Salmonella* caused the drop of about 150 milk bacterial genera to 10 in 12 hours. Interestingly, the relative abundance of genera *Bacillus* and *Macrococcus*, whose counts increased during the 12-hour incubation at 37°C (*Bacillus*-98 fold and *Macrococcus*-8000 fold), were found to be depleted in *Salmonella*-inoculated milk samples. To gain further insights into *Salmonella* survival in the iron-limited conditions of milk (due to lactoferrin), we analyzed the growth pattern of *S*. Typhimurium 4/74*Δfur* (lacking the gene encoding ferric uptake regulator) in comparison to the isogenic wild type. A lack of iron will induce the Fur-regulated siderophore production. The deletion of the fur gene resulted in a significant decline in the growth of *Salmonella* in bovine milk. Furthermore, the addition of streptonigrin and ferric chloride to milk demonstrated that inhibition of *S*. Typhimurium 4/74*Δfur* occurred due to the cytoplasmic iron accumulation and toxicity.

Significance: Our data provides preliminary insights into how *Salmonella* colonizes in milk, information that is critical to developing technologies aimed at mitigating *Salmonella* milk survival.

P3-27 Performance Evaluation of Rapid ELISA Method on Aflatoxin M1 Workflow Optimization in Dairy Products

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Introduction: Enzyme-linked immunosorbent assay (ELISA) is a fast and reliable method for aflatoxin M1 detection, but the complicated pre-treatment of dairy products has always been a challenge. Thus, it's essential to improve the detection efficiency through pre-treatment optimization while ensuring the accuracy of the kit.

Purpose: Performance evaluation of rapid ELISA method on aflatoxin M1 pretreatment and workflow optimization in dairy products.

Methods: 22 samples, under a variety of dairy categories, were analyzed after different pretreatment optimizations. Spiked solid samples (n=5, e.g.: milk powder) were directly diluted (1:10) with deionized water. pH adjustment was done for spiked recombined milk (n=5). Dilute spiked yogurt samples (n=2) with 10 mM PBS buffer (pH 7.4) at a 1:5 dilution ratio, while using skimmed milk for high viscosity and high fat content samples (n=6, e.g.: cheese and cream) under the same dilution ratio. For spiked flavored concentrated milk products (n=4), use 10 mM PBS buffer (pH 7.4) for 1:5 dilution. All samples were vortexed for 1 minute and tested directly (no centrifugation required). The recovery rate and coefficient of variation were calculated to evaluate the performance of the optimized workflow.

Results: The recovery rate of aflatoxin M1 in all the samples analyzed was within the range of 70%-120%, which was consistent with the requirements of GB Standard 5009.24-2016 that should be within the range of 50% to 120%. The coefficient of variation within each batch of sample was less than 20%, which was also consistent with the requirements of GB/T Standard 33411-2016.

Significance: The rapid ELISA method could satisfy testing requirements of aflatoxin M1 in various dairy products after pretreatment optimization, which also empowers laboratory detection efficiency.

P3-28 Adapting Statistical Process Control to *Salmonella* and Total Plate Counts in Commercial Poultry Processing

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❖ ***Developing Scientist Entrant***

Introduction: Formal statistical process control methods (SPC), like control charts, have not been widely applied to food safety. It may be possible to use SPC for controlling *Salmonella* in raw poultry because industry best practices for monitoring and preventative control include detection of this hazard directly in the product.

Purposes: To adapt statistical process control charts to *Salmonella* testing and total plate counts (TPC, indicators of quality and sanitary dress) in commercial poultry processing data.

Methods: *Salmonella* and TPC data provided by one large processor, for one facility, over about one year, was cleaned, adapted to account for assay limits of detection and quantification ranges, and then subset by processing stage. *Salmonella* and TPC data were plotted on run charts and evaluated for control chart metrics like mean (centerline), deviation (control limits) and the 8 Nelson's rules to identify special causes of variation. To identify additional metrics for SPC, classification trees were used to analyze if 28 calculated data features were associated with *Salmonella* prevalence.

Results: SPC methods identified at least 5 clusters of special causes of variation in TPC data. Two of these clusters corresponded with obvious process changes, such as a switch in wash chemical formulation, suggesting potential for SPC to identify key process shifts. Similarly, the classification tree analyses identified one significant factor associated with higher *Salmonella* prevalence, time between bird harvest and cutting into parts, that may be a new target for control.

Significance: Using SPC to detect trends in quality indicators and *Salmonella* contamination over time can reveal patterns which may be lost in the sheer volume of information, allowing for more specific corrective actions. This method can be supplemented with methods such as decision trees to identify additional external factors for control.

P3-29 Data-Mining Salmonella and Campylobacter Quantification Loads in a Commercial Poultry Processing Facility to Establish Statistical Process Control Parameters, Evaluate the Performance of Antimicrobial Intervention Schemes and Implement Risk-Based Food Safety Management Decisions

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Introduction: Bio-mapping sand monitoring can be managed and analyzed in variety of ways to examine process trends, identify reasons for changes, initiate root cause analyses for particular microbial events, and gather data on microbial performance to show inspection authorities how certain daily decisions have an impact over time.

Purpose: To implement various approaches for data analysis and visualization on pathogen bio mapping to support decision-making initiatives for food safety management in commercial poultry processing facility.

Methods: Commercial processing facility was sampled once during a 10-week period, collecting 5-samples/location/day. Ten locations were sampled: Live-Receiving (LR), Rehang (R), Post-Evisceration (PE), Pre-Chill (PRE), Normal-Post-Chill (POST), Neutralized-Post Chill (NPOST), Wings (W), Treated-Wings (TW), Tenders (T) and Treated-Tenders (TT). 50 samples were taken for each location. *Salmonella* counts and prevalence were determined using GENE-UP Quant *Salmonella* system and GENE-UP detection. Campylobacter were determined using Tempo® Campylobacter quantification system. All counts were transformed to Log CFU/sample and analyzed using R (version 4.0.4) for statistical analysis.

Results: For *Salmonella* counts there was no significant difference between shift at all locations except for Wings samples, but there was a difference between prevalence at all locations that shows a larger prevalence at second-shift except for post-evisceration, were first-shift had a higher prevalence. For *Campylobacter* counts there was no significant difference between shifts at any locations, but there was more variability between both counts and prevalence where for the first-shift LR, RH, PRE, T, W had higher counts compared to second-shifts. Prevalence was higher in the first-shift except for LR and POST when prevalence was higher in the second shift. LR and R data by flock indicate an opportunity for customized processing by flock. Log reduction from R to POST, W, TW, T and TT was 1.78, 1.28, 1.88, 1.73 and 1.85 log CFU/sample. respectively. The log difference between *Salmonella* and *Campylobacter* at the live receiving and Rehanger locations was 2.15 and 0.76 logs, respectively.

P3-30 Using Mixed Models to Assess Microbial Contamination in Raw Milk from a Pre- and Post- training Intervention in the Central Oromia Region, Ethiopia

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❖ ***Developing Scientist Entrant***

Introduction: Raw milk consumption is common among individuals from rural areas of Ethiopia, putting them at a higher risk of contracting a food-borne illness. Different risk factors, including handling practices, have been associated with poor quality and safety in the milk supply chain of the country.

Purpose: To assess microbial quality of milk pre- and post-training among smallholder farms in Central Ethiopia using a mixed modeling approach aligned with the nature of the data.

Methods: A study was conducted to assess microbial quality of raw milk before and after a training intervention conducted among smallholder female dairy farmers (n=120) from four locations in Ethiopia (Asella, Bishoftu, Holeta, Selale). Milk samples collected pre- and post-training were analyzed for total and fecal coliform using the Most Probable Number (MPN) and categorized as very low (0-20 MPN/ml), low (21-100 MPN/ml), medium (101-1000 MPN/ml) and high (>1000 MPN/ml) microbial load. Data were analyzed using Generalized Linear Mixed Models (GLMMs) assuming a multinomial distribution with ordered categories to reflect the nature of the response variable. The linear predictor included the fixed effect of training and the random blocking structure of farm.

Results: Results showed an increased probability of lower total coliform contamination in samples collected post- compared to pre-training intervention (p=0.0581). Specifically, the estimated cumulative probability of raw milk samples being at most in the low category was 12.6% (95% CI:6.9%, 22.1%) in pre-training compared to 20.6% (12.6%, 31.9%) in post-training. There was no evidence for an association of fecal coliform contamination with training (p=0.6354).

Significance: While analyzing the association between a training intervention and the microbial quality of raw milk, this study serves as an example of contemporary statistical methods that can be used when assumptions of traditional methods are not met.

P3-31 iComplai PestiPredict – Advanced Pesticide Risk Prediction for Sourced Ingredients

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Introduction: As seen in the case of ethylene-oxide where thousands of products were recalled worldwide in 2021 and 2022, the pesticides that are forbidden and typically not included in the multi-modal tests can cause completely unexpected and large-volume recalls.

Purpose: The primary objective of PestiPredict is to equip food manufacturers with a tool that can predict pesticides above the permitted maximum residue levels that are not known to exist in certain raw materials using machine learning: The so-called "unknown-unknowns".

Methods: iComplai PestiPredict integrates a robust machine learning (ML) algorithm that analyzes a comprehensive set of data including over 300 million residue testing results, over 40.000 pesticide related authority notifications worldwide and regulatory landscape. Utilizing advanced data analytics and machine learning, the system predicts the likelihood of which pesticide may appear on which raw material, for a total of app. 5.000 raw material and origin combinations, allowing for targeted and timely testing and quality control measures. The system also analyzes the gap between the user's existing test protocols and the predicted pesticides, reducing the analysis time for the user.

Results: The use of AI-infused capabilities demonstrated measurable quantitative and qualitative results: 1. Based on the raw material data availability, the accuracy rate of pesticide risk prediction reaches up to 96%. (Example: peppers from Turkey) 2. Prevention and saving of potential food recall of around €10 million. 3. The time and consequently cost saving of a risk analyst approximating around €30k per year and person.

Significance: The presented study enables food producers to switch from a reactive to proactive anticipation of potential pesticide residue risks and effectively evaluate their possible effects, thereby reducing and reducing the risk of recalls and consumer health issues. Given their significant carcinogenic potential, the control of pesticide residues is vital for safeguarding public health.

P3-32 Evaluation and Verification of WGS Bioinformatic Pipelines

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Introduction: Whole genome sequencing (WGS) is becoming more prevalent in the food industry, yet it has not been widely adopted, in large part due to the lack of standardization and expertise in data management and analytics.

Purpose: The objective of this study was to evaluate the performance and output of the CFSAN-SNP pipeline installed locally with the NCBI Pathogen Detection Isolates Browser (PDIB) to benchmark bioinformatic analyses of WGS data across tools.

Methods: Clusters of *Listeria monocytogenes*, *Cronobacter sakazakii*, and *Salmonella enterica* were chosen from the PDIB. Sequence reads and genome assemblies were downloaded from NCBI and used to run the CFSAN-SNP pipeline. The resulting SNP counts generated by the CFSAN-SNP pipeline were compared against those listed via the PDIB to benchmark the measurements obtained from the local pipeline.

Results: The outputs from the CFSAN-SNP pipeline and the PDIB matched exactly for all pairings in the *Listeria* analysis. For *Cronobacter*, the majority of the comparisons yielded identical SNP difference counts. However, three of the comparisons involving one strain resulted in a two SNP discrepancy between the CFSAN-SNP pipeline and PDIB results. For the *Salmonella* cluster, there was a higher proportion of discrepant comparisons, however, the number of SNP differences only ranged from one to- three, and the maximal SNP difference of three was only encountered for one comparison. Overall, all platforms returned highly similar results for all three species.

Significance: Given the gravity of the decisions informed by WGS analysis, it is imperative to assure the accuracy and consistency of the analytical results. This evaluation identified only slight variations across all platforms, not impacting the overall interpretation of results, giving confidence that accurate results are obtained and thus correctly informing food safety decisions.

P3-33 Evaluation of Four Key Foodborne Pathogens over Four Iterations of Healthy People

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Introduction: This presentation describes the evolution of the measurement and target-setting for four foodborne pathogens over four iterations of the Healthy People (HP) initiative, which is a national framework to promote health and reduce disease in the US through setting and tracking of objectives and targets.

Purpose: To assess four iterations of Healthy People foodborne illness data.

Methods: Since HP2000, four foodborne pathogens (*Campylobacter*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella*) have been mainly tracked using Foodborne Diseases Active Surveillance Network (FoodNet). These data span four iterations of Healthy People, from 1987 (HP2000 baseline) to 2021

(most recent HP2030 data). This presentation will detail the increasing precision used to measure the data, how the targets were established, and the progress status at the end or most recent data point of each decade.

Results: Each decade, the measurement of foodborne pathogen objectives has become more rigorous, starting with foodborne infections per 100,000 (HP2000 and HP2010), infections commonly transmitted through food (HP2020), and, most recently, laboratory-diagnosed, domestically-acquired infections (HP2030). Furthermore, the target-setting methods have also increased in precision, starting with a reduction in infections to no more than a set value (HP2000), a 50% improvement (HP2010), projection/trend analysis (HP2020) and, most recently, aligning with national programs or a percent improvement (HP2030). The progress toward these targets has been monitored and categorized (getting worse, little or no detectable change, improving, or met target) for each decade for these pathogens. These objectives have generally moved toward or met their targets each decade, with the exception of *Salmonella*, which moved away from the HP2010 target.

Significance: This presentation for the first time provides a comprehensive history of the measurement and progress of four foodborne pathogens that have been monitored over four iterations of the Healthy People initiative.

P3-34 Evaluation of Rapid Allergen Detection Method for Plant-Based Protein Beverages

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Introduction: As the consumption of plant-based protein drinks are on the rise due to health trends, it's crucial not to ignore the associated allergen risks. Enzyme-linked immunosorbent assay (ELISA) tests are the most convenient and rapid method for detecting allergens. Thus, it is necessary to verify the feasibility of ELISA test kits for plant-based protein beverages.

Purpose: Performance evaluation of ELISA methods on gliadin, peanut and walnut allergens in oat milk drinks.

Methods: Two types of oat milk samples, K1 and K2, were used for analysis. The spiking levels for each sample were ×2.5, ×5, ×7.5 and ×12.5 ppm. All samples were extracted according to the manual of the corresponding kit and the supernatant was collected after centrifugation. The recovery rate and coefficient of variation were applied to evaluate the performance of three ELISA kits (Neogen® Veratox® Gliadin R5, Veratox® Peanut and Biokits® Walnut).

Results: Oat components were not detected by these three allergen kits, indicating no cross-contamination with peanut, walnut or gliadin. The recovery rate of gluten, peanut and walnut allergen in all samples were in the range of 90%-110%, which was consistent with the requirements of GB/T Standard 27404-2008. The coefficient of variation within the batch of each sample was less than 20%, which was also consistent with the requirements of GB/T Standard 33411-2016.

Significance: The ELISA methods meet the detection requirements for multiple allergens in oat milk, which is helpful for quality control in plant-based protein beverages.

P3-35 Robustness Evaluation of a Multiplex-competitive ELISA for the Quantitation of Wheat Gluten in Fermented Dairy Products

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Introduction: There are no validated analytical methods for accurately quantifying gluten in fermented foods, primarily due to variable proteolysis and the lack of appropriate calibrants. We show a multiplex-competitive ELISA using gluten-incurred yogurt as a calibrant to accurately quantitate gluten levels in select fermented dairy products.

Purpose: To evaluate the robustness of the multiplex-competitive ELISA for quantitating gluten in select fermented dairy products.

Methods: Extraction procedure, incubation temperature, and incubation time were varied to determine the impact on the gluten quantification of 24 samples from two batches of yogurt, kefir, and buttermilk incurred with 20 µg/mL (ppm) gluten. Two incubation temperatures (25°C and 37°C), three extraction times (30 minutes, 1 hour, and 2-hours), and two incubation times (30 minutes and 1 hour) were varied in 12 different combinations. The average gluten concentration, standard deviation, recovery, and coefficient of variation were calculated for each combination. Significance of differences from the baseline values were determined by the student's unpaired *t*-test.

Results: Extraction procedure was the only parameter that significantly ($p<0.05$) affected the gluten recovery. The yogurt and kefir samples that underwent longer extraction resulted in significantly ($p\leq 0.0144$) higher gluten recoveries (107-360% average recovery), while the buttermilk samples that underwent the 1-hour extraction resulted in significantly ($p=0.0192$) lower recoveries (77-107% average recovery) compared to the baseline values (85-155% average recovery). Incubation temperature and time did not significantly ($p> 0.05$) affect gluten recoveries.

Significance: This study completed the single-laboratory validation of the multiplex-competitive ELISA and indicated that the method can be made more user-friendly by eliminating the use of an incubator and reducing the total assay time.

P3-36 Performance Verification of an ELISA-Based Crustacea Assay in Otak-otak

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Introduction: Shellfish allergy can be developed in an individual at any age and is not commonly outgrown. There are two families of shellfish- mollusks and crustaceans. Crustacean shellfish is a major food allergen and is required to be labeled on food packaging in the United States. A shellfish allergy is different from fish allergy, as the protein found in shellfish is different from fin fish. Hence, people with shellfish allergy are generally still able to consume fish. However, care should be taken due to the possibility of cross-contamination during food manufacturing. The Enzyme-Linked Immunosorbent Assay (ELISA) method is most commonly used to detect the presence of food allergens such as crustaceans.

Purpose: Performance verification of ELISA method on detecting presence of crustacean allergens in Otak-otak, a Southeast Asian fish cake made from fish mixed with spices and pounded into a paste.

Methods: 45 Otak-otak samples were sourced from supermarkets, shops and hawker centers in Singapore. The samples were homogenized in a blender and spiked at limit of quantitation (LOQ) level of 20ppb and 3xLOQ level at 60ppb. The samples were then extracted according to the test kit manual, and the recovery rate was determined.

Results: Out of the 30 samples that contained allergen information labelling in their food packaging, 28 samples were < 20ppb. For the 15 samples that were sourced from hawker centers, 12 samples were > 20ppb. The recovery of crustacea spike into the samples were between 80-120%, with RSD < 10%. The results obtained correlate well with the labelling on the food packaging. Meanwhile, although majority of the samples sourced from hawker centers were found contaminated with crustacea, allergen food labeling is not required in Singapore hawker centers. Consumers with crustacea shellfish allergy should avoid consuming otak-otak at hawker centers.

Significance: The AgraQuant® Crustacea offers a rapid and reliable tool for testing the presence of crustacean allergens in Otak-otak.

P3-37 Performance Verification of an ELISA-Based Milk and Peanut Assay from Production Sampling Points in Asian Seasoning Processing Plants

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Introduction: Cross-contamination is a common challenge faced in food manufacturing plants which could be reduced by having a thorough cleaning procedure. Food industries often rely on periodic swabbing of common food contact surfaces to test the effectiveness of their existing cleaning procedures. Rapid detection tests are prevalently used to check for presence of allergens on the environment. Common examples include lateral flow devices or

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Enzyme-Linked Immunosorbent Assays (ELISA) for a more quantitative analysis.

Purpose: Performance verification of ELISA method on detecting the presence of milk and peanut allergens on common food contact surfaces, to help manufacturers identify potential areas that are more susceptible to allergen cross-contamination in their plants.

Methods: 94 swab samples were sourced from customers in the seasoning manufacturing industry. The samples were swabbed from common food contact surfaces such as blender, mesh, filling machine, etc. using a validated swabbing kit. The areas were swabbed according to instructions in the swabbing kit manual and placed in a buffer tube. The swabs were tested for presence of milk and peanut allergens using the ELISA method.

Results: Out of the 24 samples sent for milk allergen testing, 25% of the samples were found contaminated at >20ppb, while 47% of the 30 swab samples sent for peanut allergen testing were found contaminated at >50ppb. After customers reviewed the test results, they improved their cleaning processes and sent another batch of swab samples for testing. The results for the second batch showed a significant improvement. All 15 samples tested for milk allergen were < 20ppb, and only 12% of the 25 samples sent for peanut allergen were >50ppb. The top 3 frequently cross-contaminated food contact surfaces were identified to be breaker, hopper and mesh.

Significance: The AgraQuant® Milk and Peanut and AgraQuant® Swabbing kit offers a rapid quantitative and reliable tool for testing presence of milk and peanut allergens in food processing plants.

P3-38 Performance Verification of an ELISA-Based Soy Assay on Plant-Based Food and Beverage

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Introduction: The demand of plant-based food and beverage has increased tremendously in recent years due to its health benefits and positive impact on the environment. Some ingredients that are commonly used in plant-based food and beverage are nuts, wholegrains, legumes and etc. Soybeans are a type of legumes which are good sources of protein and commonly used as meat alternatives due to its lower cost and nutritional benefits. As soybeans possess a considerable risk for consumers with soy allergy, soybeans are listed as one of the major food allergens and are required to be labeled on food packaging in the United States. Therefore, additional precaution should be taken to prevent cross-contamination in manufacturing plants that produces both soy and non-soy products in the same production line. The Enzyme-Linked Immunosorbent Assay (ELISA) method is most commonly used to detect the presence of soy allergens.

Purpose: Performance verification of ELISA method on detecting the presence of soy allergen on soy-free plant-based food and beverage that were produced in the same facilities as products that were made from soybeans.

Methods: 40 samples were sourced from customers who sent plant-based samples for soy allergen testing. The samples included plant-based seafood, plant-based chicken, plant-based beverages and etc. The samples were homogenized in a blender and spiked at limit of quantitation (LOQ) level of 40ppb and 3xLOQ level at 120ppb. The samples were extracted according to the test kit manual, and the recovery rates were determined.

Results: Soy allergen was not detected in all 40 samples that had proper food allergen labelling. The recovery rate of soy spiked into the samples were between 80-120%, with RSD < 10%. The results obtained correlate well with the labelling on the food packaging, and no soy cross-contamination was found in the samples.

Significance: The AgraQuant® Soy offers a rapid quantitative and reliable tool for testing presence of soybeans in plant-based food and beverage.

P3-39 An Evaluation of Rapid Lateral Flow Assays for Gluten Detection

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Introduction: Food allergens are proteins that can trigger an immune response in allergic individuals such as nausea, vomiting, asthma, or in extreme cases anaphylactic shock. The gluten protein, found in wheat, barley, and rye has been identified as the major causal agent in disorders like Celiac Disease. Due to this, the FDA has a regulatory limit of 20 ppm. It is thus important to have rapid test kits available for food manufacturing facilities to screen their product lines and ingredients for gluten.

Objective: To evaluate the performance of two rapid lateral flow gluten assays on various market basket products.

Methods: 22 gluten-containing and non-gluten-containing products were analyzed. Gluten containing samples were analyzed neat and diluted to extinction. Non gluten containing samples were spiked using a qualified gluten spike stock increasing in 1 ppm increments until a positive result was achieved. In addition, gluten spikes were swabbed off a stainless-steel surface to verify the detection level of a swab for each test kit.

Results: Both rapid gluten test kits performed as expected. With market basket samples, the test kits detected gluten below regulatory levels to ensure kit sensitivity within various matrices. In addition, the surface swabbing analysis indicated that the test kits can detect low levels of gluten on food contact surfaces.

Significance: The gluten free market has been booming in recent years. With diet trends and people with Celiac Disease, it is important to have an accurate test to detect gluten in food and in food manufacturing facilities.

P3-40 Development and Evaluation of a Real-Time PCR Assay for the Detection of Bovine Milk in Foods

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Introduction: The Federal Food, Drug, and Cosmetic Act lists milk as a major food allergen in the United States. This assay is designed to detect bovine milk in complex food matrices.

Purpose: To design and validate a species-specific, real-time PCR assay to detect and distinguish bovine milk in complex food matrices.

Methods: A species-specific primer/probe set was designed to target the COI mitochondrial gene for *Bos Taurus*. PCR conditions were optimized, and cross-reactivity was tested against several mammalian species. Bovine milk was spiked at known concentrations into four complex food matrices: dark chocolate, vegan cookies, dairy-free muffins, and orange juice. For comparison, beef was spiked at known concentrations into matrices as a source of more concentrated bovine DNA. Assay performance was analyzed for sensitivity and efficiency. Cross-reactivity in matrices was tested with pork spikes in chocolate and cookies.

Results: Data show reliable detection of bovine milk in all tested matrices except for dark chocolate. Internal controls confirmed the presence of PCR inhibitors in chocolate, preventing data analysis in this matrix. The LOD in all matrices is 100 ppm and the LOQ is 1000 ppm in baked goods and 100 ppm in orange juice. Efficiencies range from 100% to 118% and are matrix dependent. Comparison with data from ground beef spiking experiments indicate that low sensitivity could be attributed to low concentration of total DNA in milk. No cross-reactivity was shown in food matrices with pork spikes.

Significance: While ELISA kits are the most common detection method for milk allergens, they typically cannot distinguish a species of origin. This work validates a method to detect and distinguish bovine milk in commercial food products with high selectivity and sensitivity.

P3-41 Evaluation of Wiping Treatments for Removal of Allergens from Food-Contact Surfaces

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Introduction: Retail and food service operations use a variety of wiping methods to clean food-contact surfaces. Little information is available on the effectiveness of these methods for allergen removal.

Purpose: To evaluate wet and dry wipes on their ability to remove allergens from food-contact surfaces.

Methods: Three stainless steel (SS) and three textured white polyethylene (PE) coupons were contaminated with 0.5 g or 1 g of egg-based (powdered whole egg; reconstituted whole egg powder), gluten-containing (wheat flour; wheat-containing batter), or sesame-based (sesame flour; tahini) foods. After drying for 30 min at 23°C, coupons were wiped with either a dry paper wipe, a dry terry cloth, a wet terry cloth soaked in 200 ppm quat solution, or one, two, or three sanitizing wipes for 5 sec. After wiping treatments, coupons were tested for residual allergens with lateral flow devices (LFDs). The detection limits (LODs) for the egg, gluten and sesame LFDs were 10 mg egg, 1.6 – 3 mg gluten, and 1 µg sesame per 100 cm^2, respectively. Three independent trials were conducted for each experimental variable.

Results: LFDs detected egg, gluten, and sesame residues on most (≥ 5 out of 9) SS and PE coupons after dry wiping treatments and after the wet terry cloth treatment. Three sanitizing wipes were required to remove egg powder, reconstituted egg powder and wheat-containing batter from SS coupons, while other allergenic foods were not removed from the SS or PE coupons. There were no observable differences in the ability to remove 0.5 g than 1.0 g of food soils. LFDs were able to detect allergens on some surfaces that were visually clean.

Significance: Overall, the results indicate that it was difficult to remove the allergenic foods with most of the wiping treatments studied. Using multiple wet wipes facilitates removal of some allergenic foods.

P3-42 Evaluation of Manual and Mechanical Warewashing Treatments for Removal of Allergens from Food-Contact Surfaces

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Introduction: Food-contact surfaces used in retail and food-service operations are washed manually or with mechanical warewashers. Little information exists on the effectiveness of these wash treatments on allergen removal.

Purpose: This study evaluated the effectiveness of manual and mechanical washing methods at removing allergen-containing foods from food-contact surfaces.

Methods: Coupons made of polyethylene (PE, n=3), stainless steel (SS, n=3), and ceramic (CE, n=3) were contaminated with 0.5 g or 1 g of egg-based (egg powder; reconstituted egg powder), gluten-containing (wheat flour; batter), or sesame-based (sesame flour; tahini) foods. Coupons were manually washed (10 sec), rinsed (10 sec), and then sanitized (60 sec), in detergent solution (43°C), water (43°C), and 200 ppm quat solution (43°C), respectively. Mechanical treatments involved washing coupons in warewashing machines. Allergens on coupons were detected with qualitative lateral flow devices (LFDs). Detection limits for the egg, gluten and sesame LFDs were 10 mg egg, 1.6 – 3 mg gluten, and 1 µg sesame per 100 cm^2, respectively. Washing trials were done in triplicate.

Results: After the manual washing treatment, gluten was not detected on most (≥7 out of 9) SS coupons, while the treatment was less effective for PE and CE coupons. Egg-based foods were not detected on any of the coupons after manual washing. Mixed results were found when the manual method was used to remove sesame flour from SS and CE coupons, while complete removal was found for PE. Tahini was detected on all nine SS, PE, and CE coupons after manual washing. Mechanical washing treatments were effective at removing gluten-containing foods, egg powder, and sesame powder from all surfaces while reconstituted egg and tahini were challenging to remove.

Significance: The nature of the food soil and surface impacted washing treatment effectiveness. More extensive washing treatments are needed for some allergenic food soils.

P3-43 Development of a Mass Spectrometry-Based Non-specific Fish Detection Method for Allergen Control

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Introduction: Fish detection methods for allergen control are not broadly applicable to the diversity of fish that are commercially available.

Purpose*:* Use the selectivity inherent to MS to select target peptides that better represent the broad diversity of fish.

Methods*:* Fish (9 species) were subjected to data-dependent analysis using a Thermo Q Exactive ™ Plus Hybrid Quadrupole Orbitrap ™ MS coupled to UltiMate 3000RSL® liquid chromatography (UPLC)system (Thermo Scientific™), equipped with a Hypersil Gold C18 1.9 µ m, 100 x 1 mm analytical reversed phase column (Thermo Scientific™). Peptide identification (PEAKS ver 8.5) was performed using databases specific to each fish species. Peptide data was combined and analyzed to identify peptides that occurred in similar abundance in multiple fish species. These peptides were used in parallel-reaction monitoring (PRM) experiments to select those with desirable analytical properties. The final list of peptides was used to develop a cross-species PRM method using stable-isotope labeled peptide controls.

Results*:* Although selection of identical peptides that are present in multiple species at similar levels was difficult, the MS approach allowed the selection of analyte targets that provide a method with superior characteristics than those of commercially available fish ELISAs. The final method allowed detection of fish at 10 mg/kg in samples including incurred, processed meatballs. This method, although still sensitive to fish species (approx. 3-fold signal difference) represents a considerable improvement to commercially available ELISA in which species-dependent differences account for over 100-fold signal differences.

Significance: Fish detection for allergen control is severely hindered by lack of methods that allow detection of multiple fish species. Our novel PRM-based method represents a significant improvement in this regard.

P3-44 Food Safety, Attitude, and Practice of Food Additive, Food Allergen, and Halal Labeling Among Supermarket Consumers in Los Banos, Laguna

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Introduction: Food safety risks are faced by Filipino consumers due to inadequate and ambiguous information about the labeling of processed foods. Prolonged intake of food additives and high fructose corn syrup (HFCS) may induce obesity and metabolic dysregulation. The study described the knowledge, attitude, and practice (KAP) of food additives, HFCS, food allergens, and *halal* labeling among supermarket consumers.

Purpose: The purpose of this study is to comprehensively examine the socio-demographic background, purchasing patterns, and awareness levels of consumers regarding food additives, High Fructose Corn Syrup (HFCS), halal, and food allergens to establish connections between socio-demographic variables and consumer perceptions that influence food-related choices.

Methods: The research utilized a descriptive cross-sectional design in three Los Baños supermarkets, employing convenient sampling to survey 107 consumers. The questionnaire covered socio-demographics, purchasing patterns, and awareness of food additives, High Fructose Corn Syrup, halal, and food allergens. Data analysis involved statistical tests, including ANOVA and Kruskal-Wallis H test, to explore relationships with socio-demographic factors.

Results: The findings indicated variation in the levels of respondent's awareness showing generally inadequate knowledge, attitudes, and practices regarding food additives and HFCS. Positive notes included good food allergen knowledge but inadequate practices among allergic individuals. Halal knowledge was moderate, attitudes were unfavorable, and practices were notably poor. Associations with socio-demographic variables were explored, revealing education's significant influence across categories, while income and age had weak correlations. Positive attitudes were linked to higher income and education, and civil status affected attitudes toward food allergens and halal. Practice correlated significantly with location, civil status, and education concerning HFCS and halal.

Significance: The study highlights insufficient consumer knowledge and food label awareness, emphasizing the necessity for targeted education.

Recommendations include communication programs familiarizing consumers with various types of food label information must be developed to increase safety perceptions and respond to consumer needs on food additives, HFCS, food allergens, and halal labeling.

P3-45 International Investigation of Lead Contamination of Multi-State Apple Cinnamon Puree Pouches in 2023

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Introduction: In 2023, FDA received reports from North Carolina of children with high blood lead levels; additional exposure information and subsequent testing indicated apple cinnamon puree pouches from Manufacturer A had been contaminated with lead chromate.

Purpose: To review the investigation into this incident to identify the potential source of and contributing factors to the contamination.

Methods: FDA, states, and CDC collaborated to identify exposures to products sourced from Manufacturer A. FDA and most state laboratories used FDA's Elemental Analysis Manual method 4.7 (v.1.2) to test for lead and collected records for traceback analysis.

Results: As of 3/27/2024, FDA identified 90 confirmed complainants from 32 states and CDC identified 126 confirmed cases. FDA and states collected 193 samples of implicated product, cinnamon at import and from an on-site inspection, and various other products. Lead was detected at levels above 1ppm in 28 cinnamon apple fruit puree pouches. Regulatory actions included product recalls and import alerts. The investigation and collaboration with Ecuadorian authorities determined that cinnamon ground in Ecuador was the likely source of contamination. The leading hypothesis, supported by FDA's testing, indicated that lead chromate was likely added as an act of economically motivated adulteration.

Significance: This investigation demonstrates how lead can be introduced into food and the importance of international collaboration. Working with Ecuadorian authorities resulted in exchange of crucial information, a thorough FDA investigation of the implicated product and potential contamination routes, and collaboration with their regulatory authorities in the investigation. Food producers, particularly of children's products, should perform or require finished product and ingredient testing for lead and other heavy metals to help prevent similar issues. Spices like cinnamon are vulnerable to heavy metal contamination during the grinding stage and robust sampling of these ingredients, at the firm and import level, can aid in future detection.

P3-46 Correlations and Co-Occurrence of Arsenic, Cadmium, and Lead in Baby Foods: Evaluation of Two Statistical Approaches Adapted to Censored Data

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Introduction: Determining correlations and co-occurrence of toxic elements (TEs) in foods can help inform mitigation strategies to reduce consumer exposure.

Purpose: This study aims to evaluate and understand correlations and co-occurrence of arsenic (As), cadmium (Cd), and lead (Pb) in baby foods.

Methods: TE data for baby foods collected in the US from FDA's Toxic Elements Program (FY2008-2023) and two special FDA surveys (FY2013-2014 and FY2021) were compiled. Co-occurrence was assessed by calculating the frequency of samples having detectable contaminant levels for TE pairs (i.e., As-Cd, As-Pb, Cd-Pb) and As-Cd-Pb. Pairwise TE correlations were evaluated using two statistical approaches adapted to censored data: (i) non-parametric Kendall's tau and (ii) parametric Bayesian modeling. The impacts of theoretical changes in the distribution of one TE in baby foods on the concentration distributions of other TEs were assessed.

Results: In total, 1,246 baby food samples were included in this study, with 1,117 samples tested for ≥2 of the TEs, and 1,113 tested for all 3 TEs. Most samples had detectable levels of TEs, reflecting low limits of detection (LOD), where the median LODs were 0.6 ppb, 0.1 ppb, and 0.8 ppb for As, Cd, and Pb, respectively. Parametric and non-parametric correlation analyses showed significant correlation coefficients, with consistency in the directionality and relative strength of these correlations. The parametric method had more discriminatory power than the non-parametric method but required the assumption of multivariate normality for the logarithmic concentration distribution. Generally, reducing levels of one TE will reduce levels of the other TEs.

Significance: This study helps inform risk managers on strategies to reduce TE exposures in baby foods.

P3-47 Total Diet Study-Based Estimates of Children's Exposures to Lead and Cadmium in the U.S.

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Introduction: Exposure to lead (Pb) and cadmium (Cd) can occur through many sources, including food, and can cause serious health effects. Reducing exposure to toxic elements especially for babies and children is one of FDA's highest priorities. We published an assessment on dietary exposure to Pb and Cd in children aged 1-6 years in 2019.

Purpose: To update the estimation of children's total dietary exposure to Pb and Cd based on newly published data and identify key dietary sources of exposures.

Methods: Dietary exposure estimates to lead and cadmium for children 1-6 years were updated using U.S. FDA's Total Diet Study (TDS) 2018-2020 concentration data and food intake amounts reported in the 2017-2018 National Health and Nutrition Examination Surveys/What We Eat In America (NHANES/WWEIA). NHANES/WWEIA foods were mapped to TDS foods using ingredient percentages. Estimated mean and 90th percentile exposures were compared with FDA's published Interim Reference Value (IRL) for lead and FDA's Toxicological Reference Value (TRV) for cadmium. Leading food groups contributing to children's lead and cadmium exposures were identified.

Results: Total dietary exposure estimates using updated concentration and intake data and mapping based on ingredients were comparable to those published in 2019. Lower bound (LB) mean and 90th percentile lead exposures (non-detects set to zero) were slightly lower than those published earlier and still below FDA's IRL. Estimated LB mean and 90th percentile cadmium dietary exposures slightly increased since those published in 2019 and continued to be above the TRV. Consistent with earlier findings, the food group contributing most to the updated lead and cadmium exposure estimates was grains and baking (e.g., baked products, flour, baking powder).

Significance: The results of this study can be used to inform FDA's Closer to Zero on the public health impact of children's dietary exposures to Pb and Cd and identify actions for mitigation.

P3-48 Development of a Smartphone-Integrated Microfluidic Paper-Based Optosensing Platform for *In-Situ* Detection of Histamine in Canned Tuna

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Introduction: Histamine is a biogenic amine that functions to transmit signals between cells in various organs of the immune system, including the skin and gut. Over consumption of histamine in foods may result in histamine poisoning or scombroid poisoning, resulting in symptoms such as nausea, headache, diarrhea, and asthma.
Purpose: A smartphone-integrated microfluidic paper-based optosensing platform was developed in this study for in-situ detection and quantification of histamine in canned tuna.
Methods: Molecularly imprinted polymers (MIPs) were synthesized via precipitation polymerization and utilized as the dispersive solid phase extraction (d-SPE) sorbent to selectively extract histamine from canned tuna. Carbon quantum dots (CQDs) with the particle size of 3.06 ± 0.26 nm functioning as a fluorescent probe were synthesized and introduced onto the microzones of the microfluidic paper device. This facilitated a linear ratiometric response along with a noticeable fluorescence color change from dark red to vivid blue upon the addition of histamine. The change in fluorescence on the paper device was converted into specific RGB values using a portable UV light box combined with a smartphone.
Results: This assay achieved the limit of detection of 14.04 mg/kg with the linear range from 20-100 mg/kg of histamine in canned tuna, meeting the requirement of maximum residue level set in both Europe and North America. The entire molecular imprinting-microfluidic optosensing test could be completed in 45 min including sample preparation.
Significance: This developed method has the potential for practical applications in rapid determination of histamine in agri-foods.

P3-49 Multispectral Sorting to Reduce Aflatoxins in Bulk Maize Retains Some Efficacy when Tested under Less Controlled Field Conditions

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✦ ***Undergraduate Student Award Entrant***

Introduction: Multispectral sorting calibrated to remove visually high-risk maize kernels can remove highly mycotoxin contaminated single maize kernels from bulk maize streams, reducing aflatoxin or fumonisin levels when present. This sorting process generalizes across countries (calibration to maize from Ghana maize works for maize from Kenya). But so far, all sorting has been done under well-controlled laboratory conditions.
Purpose: This study tests the robustness of the multispectral sorting process under less-controlled conditions.
Methods: 45 maize samples were collected from local Kenyan markets. Unlike previous studies, all samples (not just toxin positive samples) were sorted to remove kernels visibly at high risk of mycotoxin contamination- including moldy, broken, or insect infected ones. This sorting was done in a field laboratory of the University of Nairobi, using a Ghanan maize algorithm to sort Kenya maize kernels. After shipping to Illinois, a stainless-steel grain mill was used to grind 20-40g sorted maize samples, 5g subsamples from each of the 45 maize samples were extracted at 1:5 w/v in 80% methanol, and total aflatoxin analyzed by ELISA.
Results: The level of aflatoxin (always in log(ng/g)) in the accept stream ranged from 0.04 to 1.80 with a mean of 0.17. The reject stream ranged from 0.04 to 3.53 with a mean of 0.35. The aflatoxin levels were significantly (p=0.04) reduced in the accept compared to reject stream, by a mean of 0.3, and 43/45 (95%) of accepted samples (compared to 39/45 rejected samples) were below the Kenyan regulatory threshold of 10 ng/g, indicating useful food safety outcomes.
Significance: A mycotoxin intervention that worked in well-controlled lab experiments does still for reducing mycotoxin levels under less-controlled field conditions.

P3-50 Detection of Mycotoxins with Confidence – Overcoming Interferences in Challenging Matrices

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Introduction: Mycotoxins are toxic compounds which are produced naturally by certain types of molds which can grow on numerous foodstuffs such as cereals, dried fruits, nuts, and spices during storage often under warm, damp, and humid conditions. Most mycotoxins are chemically stable and survive food processing. Exposure to mycotoxins can happen either directly by eating infected food or indirectly from animals that are fed contaminated feed. Mycotoxins because of their toxicity to humans and animals are regulated by various international, national, and regional regulations in foods and feeds.
Purpose: The fast methods of analysis of multiple mycotoxins of high sensitivity and specificity are required for evaluation of food and feed safety, regulatory compliance, and quality control purposes. The conventional mass spectroscopic and other methods of Mycotoxin's analysis involve complex and time-consuming extraction methods and suffer from matrix interference issues.
Methods: We performed UHPLC-ESI-MS/MS analysis of various mycotoxins extracted by simple extraction from various mycotoxin spiked matrices including wheat samples. The matrix interference was observed particularly in citrinin analysis. We similarly analyzed multiple mycotoxins in different matrices by UHPLC-ESI-QTOF-MS analysis after simple extraction. The analyses were performed in spiked wheat samples and contaminated red chili samples employing Bruker's VIP-HESI source as well as a conventional ESI source.
Results: QTOF-MS provided satisfactory analysis of evaluated mycotoxins without any matrix interference. The high sensitivity and selectivity of QTOF-MS makes the method straight forward without a need for any complex sample preparation. The use of VIP-HESI in place of a conventional ESI source improved the sensitivity of the analysis from 3 to 13-fold for different mycotoxins.
Signficance: Multiple mycotoxins analysis by UHPLC-ESI -QTOF-MS employing VIP-HESI source and simplified extraction provided a good quality of analysis for the evaluated mycotoxins with no matrix interference.

P3-51 Knowledge of Conventional Food Consumers about Pesticides: How It Can Impact Food Safety and Guide Risk Communication?

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Introduction: Pesticides are a chemical hazard that negatively impacts food consumers' health.
Purpose: To assess the consumers' knowledge about food contamination with pesticides and possible methods to remove pesticides from food.
Methods: 94 consumers from street markets were interviewed using a questionnaire containing nine questions about sociodemographic characteristics and eight questions about the knowledge of food contamination with pesticides and possible methods to remove pesticides from food. The answer options for the knowledge questions were "yes", "no", or "I do not know", and the correct answer changed between "yes", and "no". One point was given for correct answers and zero points for incorrect or "I do not know" answers. Data were evaluated by the percentage of correct answers and a final knowledge score ranging from 0 to 8 points. We tested the correlation between knowledge and sociodemographic characteristics using Jamovi 2.3.21. The Brazilian Research Ethics Committee approved the project under the number 6.074.703.

Results: From the 94 participants, the majority were women (55.3%), ranging between 17-29 years old (41.5%), white ethnicity (71.3%), with a high education level (56.4% had a university degree), and with a monthly income of 3-10 minimum wages (51.1%). The average knowledge score was 5.9 (Standard deviation=1.98), demonstrating an average knowledge about consuming foods that potentially contain pesticide residues. The questions with the lowest percentage of correct answers (45.7 to 75.5% - insufficient to medium) addressed systemic pesticide removal through food sanitization using bicarbonate, vinegar, and hypochlorite. The knowledge score was not correlated (p>0.05) with sociodemographic characteristics.

Significance: Consumers of conventional foods have an average knowledge regarding food contamination with pesticides and possible methods to remove it from food. Knowledge about removing systemic pesticides from food by sanitization with bicarbonate showed the lowest percentage, which may guide education and risk communication in public health.

P3-52 Characterization of a Low-Moisture Food Persistent Bacterial Population (LMF PBP) and Impacts of Nutrient Type and Relative Humidity

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Introduction: Moisture in dry processing plants poses significant contamination risks, resulting in persistent bacterial populations (PBPs) and recalls low-moisture foods (LMFs). Impacts of nutrient type and environmental conditions on LMF PBPs are not well understood.

Purpose: This study investigated the survival of *Salmonella* Tennessee LMF PBP on stainless steel coupons within three LMFs (nonfat dry milk powder (NFDM), peanut butter powder, and chicken powder) and a nonfood matrix (silicon dioxide, SiO_2) that were stored at 50% relative humidity (RH).

Method: Using a previously established method to form an LMF PBP, a mold was used to distribute one of the three powders (0.5 g) on wax paper; and the coupons (inoculated with 0.1 ml *Salmonella)*, were inverted and pressed on the wax paper to pick up the powder. After drying, coupons were stored at varying temperatures (25-35°C) and relative humidity level (RH, 50%), reflecting LMF processing conditions. Salmonellae were enumerated from LMF PBPs on the coupons on predetermined dates (0-28 days). Each experiment was conducted in triplicate, means and standard deviation were calculated, and two-way ANOVA and Tukey's HSD were used for analyses.

Result: For coupons stored at 50% RH, *Salmonella* LMF PBP reduced by the following: NFDM, 1.6±0.1 log CFU/cm^2; SiO_2, 1.6±0.1 log CFU/cm^2; peanut butter powder, 1.8±0.1 log CFU/cm^2; chicken powder, 2.7±0.4 log CFU/cm^2; and no matrix, 4.8±0.6 log CFU/cm^2. Analyses showed that populations reduced significantly less in NFDM, SiO_2, peanut butter powder and chicken powder food matrices compared to *Salmonella* without an LMF PBP matrix (p<0.05).

Significance: Nutrient type and environmental conditions, such as RH, impact pathogen survival in LMF PBPs, which has great implications for LMF processors who seek to prevent and eliminate harborage of pathogens in dry manufacturing environments.

P3-53 Evaluation of Hygiena's BAX® System Real-Time PCR Assays for the Detection of *Salmonella* and *Listeria* from Large Test Portions of Almond Butter and Peanut Butter

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Introduction: Microbial contaminants have been associated with various nuts and/or nut butters. The low water activity and high-fat environment provide a beneficial effect to pathogens by protecting cells and increasing strain thermal tolerance. Therefore, cells can survive for extended periods of time even when pasteurization or high-temperature treatments are used during processing. This presents a significant food safety risk, as these products have a long shelf life and are sold as ready-to-eat.

Purpose: In these studies, the performance of two real-time PCR assays was compared to the FDA BAM reference method for the detection of *Salmonella* and *Listeria* in almond butter and peanut butter, respectively.

Methods: Bulk portions of almond butter were inoculated with *Salmonella*, and bulk portions of peanut butter were inoculated with *Listeria* to create low (0.5-2 MPN/test portion) and high (5-10 MPN/test portion) levels of contamination. Samples were blended for uniform distribution, equilibrated for 2 weeks and enumerated right before use. Unpaired samples were then prepared for each test method (*Salmonella* 375 g, *Listeria* 125 g) and the appropriate FDA BAM method (25 g) using different pre-enrichment protocols. Test method samples were analyzed by real-time PCR and all samples were confirmed by culture.

Results: For almond butter, *Salmonella* was detected in 3/20 low-inoculated samples and 5/5 high-inoculated samples after a 3-hour BHI regrowth. For peanut butter, *Listeria* was detected in 16/20 low-inoculated samples and 5/5 high-inoculated samples. All test method results were identical to culture. The probability of detection (POD) between the test and reference method showed no significant differences for any organism tested.

Significance: Overall, the BAX® System Real-Time PCR assays are validated fit-for-purpose with statistical equivalence to the reference method for the detection of *Salmonella* in 375 g samples of almond butter and *Listeria* in 125 g samples of peanut butter.

P3-54 Matrix Validation of 375 g of Romaine Lettuce for the Detection of *Listeria* Using Hygiena's BAX® System

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Introduction: Produce has been responsible for numerous foodborne outbreaks affecting a diverse array of fruits and vegetables. Recently, there have been rising concerns over *Listeria monocytogenes* in the produce industry.

Purpose: The typical industry standard for the analysis of *Listeria* uses either a 25g or 125g analytical test portion. To prepare and better align to pre-harvest and post-harvest pathogen testing for *E. coli* O157:H7, STEC, and *Salmonella*, a validation study was planned for the analysis of *Listeria* in larger 375 g analytical test portions.

Methods*:* Romaine lettuce was artificially inoculated with *L. monocytogenes* at two levels: a low level to obtain fractional recovery and a high level to obtain all positives. Samples were held at 4 °C for 72 hours and then unpaired samples were divided into 375 g test method and 25 g FDA BAM reference method portions. Test method samples (n=30) were enriched with 2,250 mL pre-warmed (35 °C) Actero *Listeria* media and incubated at 35 °C for 22-24 hours. Reference method samples (n=30) were enriched following the FDA BAM Chapter 10 protocol.

Results*:* Test method samples were analyzed by real-time PCR and returned fractional positive results for 9/20 low-inoculated samples and all positive results for 5/5 high-inoculated samples. All samples were confirmed by culture with complete agreement. Test and reference results were compared using the probability of detection (POD). No significant difference was determined, as the 95% confidence interval contained zero for all levels.

Significance: Overall, the BAX® System method is accurate and reliable for the detection of *Listeria* in 375 g samples of romaine lettuce, equivalent to the FDA BAM reference method.

P3-55 Process Validation on the Baking of Sugar Waffles: From Lab to Industrial Scale

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Introduction: Heat treatments are a widely used and effective method for inactivating microorganisms in the food industry. However, heat inactivation is influenced by several factors, including strain resistance, food matrix, temperature-time profiles, and preculturing conditions. To ensure the safety of heat-treated food products, industrial validation is crucial. This validation utilizes a surrogate strain, a non-pathogenic microorganism with heat resistance equivalent to the most resistant pathogen associated with the specific food product.

Purpose: The objective is to assess the suitability of the surrogate *strain Enterococcus faecium* NRRLB-2354 for validating the inactivation of *Salmonella* spp. in sugar waffle production under industrial conditions.

Methods: A laboratory-based assessment is conducted to compare the heat resistance of a surrogate strain *Enterococcus faecium* NRRLB-2354 and *Salmonella* spp. cocktail (*Salmonella* Montevideo, *Salmonella* Thompson, and *Salmonella* Enteritidis) during the baking of the waffles at an industrial-relevant time-temperature combination by inoculating the flour (n=3). The baked waffles are plated out on semi-selective media: TSA with an overlay of Kanamycin aesculin azide agar (KAA agar) or XLD agar; the overlayer is applied after 4 hours of incubation at 37°C. Also, an enrichment is performed in 25 g of the waffles in KAA or BPW at 37°C for 48 hours. Finally, the surrogate strain is applied in the industrial baking process of waffles in a Belgian company, evaluating the inactivation efficacy of their process (n=3).

Results: Laboratory-based analyses revealed no plate counts of both the surrogate and pathogenic strains. Enrichment results confirmed a comparable heat resistance of *Enterococcus faecium* NRRLB-2354 and the *Salmonella* spp. cocktail. In the industrial setting, the surrogate strain effectively demonstrated the process's efficacy.

Significance: *Enterococcus faecium* NRRLB-2354 is an appropriate surrogate strain to validate the inactivation of vegetative food pathogens during the baking of sugar waffles allowing the industry to guarantee the microbial safety of their products.

P3-56 Photothermal Inactivation of *Salmonella enterica* in Paprika Powder by Ultra-High Irradiance Blue (405 nm) Light

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Introduction: The control of *Salmonella* contamination in spices represents a significant challenge due to its tolerance to desiccation stress and enhanced resistance to traditional thermal treatments. Ultra-high irradiance (UHI) blue light-emitting diodes (LEDs) have emerged as a novel intervention to inactivate foodborne pathogens by photochemical and photothermal means.

Purpose: To determine the inactivation kinetics of *Salmonella* spp. in paprika powder treated with UHI blue light (405nm) and to evaluate the impact of these treatments on selected paprika quality attributes.

Methods: Paprika samples (n=4) were inoculated with a 5-strain *Salmonella* cocktail, acclimated for 7 days (22 °C; 0.55 a_w), and irradiated with 405 nm LED at a fixed distance (5 cm) using different irradiances (548, 697, and 842 mW/cm^2) and exposure times (60, 120, 180, and 240 s). The survival curves of *Salmonella* were mathematically described using a Weibull model. Moisture content, surface color and extractable color of paprika were analyzed according to standard methods.

Results: Irradiation treatments resulted in a significant increase (p<0.05) in surface temperature, with values reaching up to 131.7 °C after 240 s at 842 mW/cm^2. This photothermal effect caused a significant decrease (p<0.05) in *Salmonella* counts, with reduction levels ranging from 2.1 to 7.8 logs after 240 s. The inactivation of *Salmonella* did not follow first-order kinetics, but a modified Weibull model with irradiance-dependent parameters satisfactorily described the inactivation kinetics. The moisture content and color profile of paprika samples were significantly affected (p<0.05) by the irradiance level and exposure time. Color degradation rates increased with increasing process temperature.

Significance: The obtained results indicate that UHI LED technology could serve as an alternative dry pasteurization method to improve the safety of spices. These findings are thus important in supporting the development and optimization of LED technology for food safety applications in the food industry.

P3-57 Thermal Inactivation of *E. faecium* and *Salmonella* in Oatmeal Cookies with Raisins: Impact of Inclusion

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Introduction: This study investigated how raisin inclusion location in oatmeal cookies affects thermal inactivation rates of *Enterococcus faecium* and *Salmonella* during baking, by examining cookies with raisins distributed throughout, localized centrally, or on the surface only.

Methods: Oatmeal cookies were prepared with raisins incorporated throughout the entire cookie dough, localized to the center, or on the surface only. Cookies without raisins were used as controls. Samples were inoculated with approximately 7 log CFU/g of *E. faecium* or a cocktail of *Salmonella* strains and baked at 350°F in an isothermal oven. Survival was evaluated by plating at 3 min intervals during a 15 min baking period.

Results: In cookies without any inclusions, baking at 350°F for 12 and 15 minutes resulted in reductions of *Salmonella* by 4.95 and >7.56 log, respectively. For cookies containing raisins as an inclusion, baking at 350°F for 12 minutes led to a 6.70 log reduction in *Salmonella*. When raisins were incorporated either in the center or on the surface of the cookies, *Salmonella* was reduced by 6.60 and 6.58 log, respectively, after baking at 350°F for 12 minutes. *E. faecium* was significantly more thermotolerant than *Salmonella* in oatmeal cookies both with and without raisins.

Significance: These findings highlight the efficacy of the baking process in reducing *Salmonella* in oatmeal cookies, with the presence of inclusions. The data also demonstrate suitability of *E. faecium* as a surrogate microorganism for thermal process validation of oatmeal cookies with raisins. Additionally, the data showed raisins did not pose risk from a microbiological inactivation perspective, as inclusions received sufficient lethality with greater than 5 log reductions of *Salmonella* during baking.

P3-58 *Enterococcus faecium* as a Bacterial Surrogate for *Salmonella* Inactivation during Red Chile Drying

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Introduction: *Salmonella enterica* has been responsible for foodborne disease outbreaks in a wide range of foods including dried chile peppers. Also, there have been numerous recalls of chile peppers over the last few years because of *Salmonella* contamination. Since dried chile pepper processing is an important part of the New Mexico Food Industry, we evaluated drying procedures used in the industry for their efficacy of inactivation of the pathogen.

Purpose: To evaluate the heat treatments used during commercial drying of red chile in New Mexico drying operations for *Salmonella inactivation*.

Methods: *Enterococcus faecium (*NRLL B-2354) and five strains of *Salmonella enterica* that were isolated from New Mexico chile (NMSU 169, S27, 266, 264, 416) were the test organisms used. Red chile pods were inoculated with a five-strain cocktail of *S. enterica or E. faecium* NRRL B-2354. Inoculated peppers were heated in a lab scale drier from temperatures ranging from 140°F to 180°F (60°C – 82°C) , and survival evaluated by plating on split plates (TSAYE/XLD for *Salmonella* and TSA/YE for *Enterococcus*). Additionally, peppers inoculated with *E. faecium* were dried in commercial drying facilities and survivors enumerated after drying.

Results:. In lab trials at 160°F, the D value for Enterococcus 67.1 min while that of *S. enterica* was 9.6 min. The time temperature combinations used

during commercial drying in 5 facilities in New Mexico showed 4.0 to 6.2 log reductions in *E. faecium* on inoculated red chile peppers after drying. These results show that the heat treatments used during the manufacture of commercially processed dried red chile were sufficient to result in a five-log reduction in *Salmonella*. *Enterococcus faecium* was a suitable surrogate for *Salmonella* for chile drying.

Significance: The *Salmonella* inactivation achieved during red chile drying in the New Mexico commercial operations exceeded that required in the Food Safety Modernization Act and showed that the processes examined were adequate to ensure safety of the product.

P3-59 The Survival of Listeria monocytogenes on Dried Gala Apple: Influence of Water Activity, Storage Temperature

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❖ *Developing Scientist Entrant*

Introduction: *Listeria monocytogenes* can endure prolonged periods in dry conditions, yet there is a general lack of understanding about its survival on dried apple products and the factors influencing its persistence in these products.

Purpose: This study aims to explore the impact of storage conditions, including water activity (a_w) and temperature, on the survival of *L. monocytogenes* on dried apples during extended storage.

Methods: Dried apple slices were inoculated with ~9 $\log_{10}$CFU/g 3-strain *L. monocytogenes* cocktail using a dry inoculation method. The inoculated apple slices were equilibrated to targeted a_w of 0.25 ± 0.02 and 0.45 ± 0.02 in equilibrium chambers, respectively, then packed in moisture-barrier bags, and stored at 4 °C and ambient temperature. The survival of *L. monocytogenes* was quantified throughout the storage period. Three 1g dried apple slices were sampled at each sampling point. Data were analyzed by one-way analysis of variance (ANOVA) and followed by Tukey's test using IBM SPSS 26 and reported as mean ± SEM, P <0.05 were considered significant.

Results: L. monocytogenes exhibits greater stability in desiccated Gala apple slices compared to Granny Smith apples. Its resistance in dried apples is significantly impacted by a_w, particularly at ambient temperature. In desiccated Gala apple slices stored at ambient temperature, a 3.98-4 log reduction was observed at a_w of 0.25 over 48 weeks, compared to a 5.04-7.08 log reduction at a_w of 0.45. At 4°C, *L. monocytogenes* maintained a relatively stable population over 48 weeks of storage, with 1.48-1.76 and 1.76-1.95 log reduction, respectively, suggesting that a_w has a diminished impact on the survivability of *L. monocytogenes* at lower temperature.

Significance: This study provides valuable insights into the impacts of apple variety, aw, and storage temperature on *L. monocytogenes* stability in desiccated apple slices, offering a scientific basis for enhancing food safety management.

P3-60 Developing and Investigating *in vitro Cronobacter sakazakii* Dry Surface Biofilms from Environmental and Clinical Isolates

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Introduction: Cronobacter sakazakii contamination was responsible for the 2022 powdered infant formula (PIF) food safety and security crisis in the United States impacting millions of infants. There is increasing evidence that pathogens, including C. sakazakii, can exist for extended periods of time in low-moisture environments such as dry or desiccated biofilms, underscoring a need for improved mitigation strategies.

Purpose: This study aimed to develop an *in vitro C. sakazakii* dry surface biofilm model to better understand adaptability to low moisture environments and for future exploration of sanitation strategies.

Methods: Single-species biofilms of *C. sakazakii* ATCC 12868 and three clinical isolates were developed in the CDC Biofilm Reactor® on borosilicate glass coupons based on the EPA MLB SOP MB-19 protocol. Biofilms were harvested and desiccated for 24, 48 and 72 h at 21°C to form dry-surface biofilms (DSB). Coupons were fixed with formaldehyde for subsequent immunostaining and imaging with confocal laser scanning microscopy (CLSM). Testing was done in triplicate and statistical analysis was completed in SAS.

Results: There were statistically significant differences among strains' ability to form and survive as a DSB ($p<0.05$), with higher cell densities formed from blood and cerebral spinal fluid isolates compared to a trachea isolate and *C. sakazakii* ATCC 12868. Average cell density for wet surface biofilms (0 h) exceeded five $\log_{10}$ for all samples, which were statistically significantly higher than all subsequent time points ($p<0.05$). CLSM images confirmed biofilm development and that the biofilm structure remained over time.

Significance: C. sakazakii can form a biofilm in vitro and cells remain viable over time. This work is inceptive toward the larger goal of multi-species biofilms for sanitation strategy development for LMF systems.

P3-61 Mitigating *Salmonella* Contamination in Pizza Dough via Cold Plasma-Based Hurdle Intervention and Evaluating Its Influence on Pizza Base Quality

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❖ *Developing Scientist Entrant*

Introduction: Over the last decade, there has been a rise in the occurrences of foodborne illness outbreaks and recalls linked to wheat flour and its products, due to *Salmonella* contamination. Recent events, such as the 2022 French outbreak and the 2023 pizza recall, vividly illustrate the risks involved, as investigations have consistently traced the source of the pathogens back to wheat flour. This emphasizes the persistent threat posed by these harmful microorganisms in products containing wheat.

Purpose: To investigate the survival of *Salmonella* after cold plasma-based treatment.

Methods: Organic wheat flour was inoculated with *Salmonella* (cocktail ~7 log CFU/g) and was dried to its pre-inoculated weight. Subsequently, the wheat flour was exposed to plasma generated in the air for 15 min. The pizza dough prepared using plasma-activated water (PAW) was further subjected to in-package cold plasma treatment (generated in atmospheric pressure air at 30 kV) to extend its shelf life. The reduction of the pathogenic load was evaluated by plating appropriate dilutions on xylose lysine deoxycholate agar (XLD) plates with a tryptic soy agar (TSA) overlay.

Results: The study's findings revealed that wheat flour treated with atmospheric cold plasma at 21 kV/6 min showed a notable reduction of 2.08 log CFU/g ($P<0.05$) in *Salmonella* load. Additionally, incorporating plasma-activated water in pizza dough formulation led to a reduction of at least 0.87 log CFU/g ($P<0.05$). Furthermore, in-package cold plasma exposure of packaged pizza dough further contributed to a reduction of 0.94 log CFU/g ($P<0.05$) in *Salmonella* load. The collective implementation of these hurdle interventions resulted in a combined reduction of 3.91 log CFU/g in *Salmonella* contamination. Additionally, cold plasma treatment did not significantly alter the physico-chemical properties of pizza base.

Significance: The findings of this study could be leveraged to develop more effective methods aimed at enhancing the food safety of pizza dough and preventing *Salmonella* contamination.

P3-62 Effect of Oil Exposure on the Heat Resistance of *Salmonella enterica* Serovar Enteritidis Phage Type 30 in Roasted Peanut Products

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Introduction: Several studies have shown that the heat resistance of foodborne pathogens in low-moisture foods (LMFs) is largely influenced by the water activity and oil content.
Purpose: This study aimed to understand the heat resistance (D-value) of *Salmonella* in roasted peanut products with different particle sizes, and to clarify the difference of protective effect of different oil exposure on *Salmonella*.
Methods: In this study, roasted peanut products with different particle sizes were selected as different degree of oil exposure LMF models. For example, the degree of oil exposure of 10-mesh peanuts (0.85-2.00 mm), 20-mesh peanuts (0.43-0.85 mm), and 40-mesh peanuts (0.43-0.85 mm) was 22.13%, 38.56%, and 43.40%, respectively. *Salmonella enterica* Enteritidis Phage Type 30 (S. Enteritidis) was inoculated into peanuts with different particle sizes (10-mesh, 20-mesh, 40-mesh). It was isothermally treated to obtain heat resistance parameters ($a_{w,25℃}$ = 0.32 ± 0.02).
Results: The results showed that heat resistance of *Salmonella* increased with increasing oil exposure over a range. For instance, the $D_{80℃}$ in 10-mesh peanuts was 67.19 min, the $D_{80℃}$ in 20-mesh peanuts was 83.83 min, the $D_{85℃}$ in 10-mesh peanuts was 20.81 min, and the $D_{85℃}$ in 20-mesh peanuts was 47.99 min. However, the heat resistance of *Salmonella* decreased with further increase in oil exposure, the $D_{80℃}$, $D_{85℃}$ in 40-mesh peanuts was 52.19 min and 33.98 min.
Significance: We confirmed that different degrees of oil exposure had different protective effects on *Salmonella*. But the relationship between heat resistance of *Salmonella* and oil exposure degree did not show a completely consistent change, its related mechanism needs further study.

P3-63 Mitigating Mushroom Risks: Evaluating Cooking Practices for *Salmonella* Reduction in Dried Mushrooms

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Introduction: With an influx of dried mushroom products on the market and incorporation into recipes, recalls and outbreaks of Salmonella enterica have been noted, calling into question the safety and best practices of handling of these products.
Purpose: This study determined the inactivation of *Salmonella* Stanley on dried mushrooms when prepared by three different preparation procedures commonly used by consumers.
Methods: Sliced and powdered wood ear mushrooms were inoculated with *Salmonella* Stanley and equilibrated for 2 days at 50% relative humidity. Inoculated sliced (10 g) and powdered mushrooms (3 g) samples were subjected to three preparation methods: 1) Sliced mushrooms treated with 80°C water (90 mL) and left to cool naturally for 10 min, 2) Sliced mushrooms soaked in tepid water (20°C, 90 mL) for 30 min and treated for 10 min with 100°C water, and 3) Powdered mushrooms treated with 80°C water (230 mL) and left to cool naturally for 10 min. Samples were removed at predetermined time intervals, plated onto selective and differential agar, incubated, and enumerated to determine log reductions. The experiment was replicated three times. ANOVA and Tukey's pairwise comparisons were conducted to determine statistically significant differences between mean reductions.
***Results*:** Sliced mushrooms soaked in tepid water and treated with 100°C water resulted in significantly higher log reductions at each time point compared to the other two treatments (p<0.05), yielding a 5-log reduction of *Salmonella* after only 2 min. Sliced mushrooms that underwent treatment in 80°C water demonstrated a 5-log reduction of *Salmonella* after 10 minutes. A maximum of 4-log reduction of *Salmonella* was observed for powdered mushrooms treated in 80°C water.
Significance: This study provides preparation recommendations to reduce Salmonella spp. populations in dried mushrooms.

P3-64 Controlling Mold Spoilage in Sugar-Free Jellies, Reducing Spoilage in the Supply Chain

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Introduction: Mold is the main microbiological deterioration problem for jelly producers. More than most other food spoilage organisms, molds are highly tolerant to salt and sugar. Although most molds prefer warmer temperatures, they can also grow under refrigeration conditions. Therefore, knowing the targets of antimicrobial or antifungal issue is essential to prescribe appropriate antimicrobial agents.
Purpose: To investigate the impact of dry vinegar on mold outgrowth in sugar free jellies.
Methods: Mold species were isolated from several batches of sugar free jellies, spoiled during their shelf life or due to packaging defaults. The strains were sequenced using ITS sequencing and identified as *Penicillium glabrum*, and *Penicillium crustosum*. Standard Jelly (pH 3.38, Brix° 0.1) and Jelly containing 0.16%, Provian NDV) was inoculated with individual strains at 4-log CFU/g mold (*Penicillium* sp.), 4-log CFU/g and 2-log CFU/g mold cocktails. All samples were assayed by daily visual observation for two months, and then weekly observations for up to six months (@ 25°C and 15°C). Uninoculated samples were assayed for pH and Brix at Day 0.
Results: Provian NDV 0.2% prevents the growth of *Penicillium sp.* mold on jellies. A typical increase in shelf life of over average of 150% (3-day increase) over regular jelly @25°C and 29%[(2-day increase) @15°C)] (P<0.05). Mold growth is clearly inhibited by Kerry's neutralized dry vinegar, as seen for both single strains and cocktails at various levels of inoculation. Neutralized dry vinegar achieves a 6-month mold-free shelf life at concentrations lower than 0.2%, making it a useful ingredient for preserving and extending the shelf life of jelly.
Significance: The study provides results to support the use of a neutralized dry vinegar to prevent and inhibit mold growth in jellies by helping producers reach their shelf life, cost reduction, and sustainability goals.

P3-65 Controlling Yeast, Acetic and Lactic Acid Bacteria Spoilage to Increase Shelf Life in Hard Seltzers Using Smokes

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Introduction: Hard seltzers are high in nutrients, water activity (aw) and are prone to microbial spoilage, especially with yeast, acetic acid bacteria (AAB) and lactic acid bacteria (LAB).
***Purpose*:** To investigate the impact of pH, alcohol levels and Kerry smoke antimicrobials on the rate of spoilage of common beverage yeast, AAB and LAB strains in different hard seltzers with varying alcohol levels
***Methods*:** Hard seltzers were prepared to varying pHs, alcohol levels (4-8%) with Kerry smoke (0-1% dosage) compared to sorbate (0.3%) and Yeast strains tested*: Zygosaccharomyces bailii, Saccharomyces cerevisiae*; AAB strains: *Acetobacter aceti*, *Acetobacter pasteurianus*;, LAB strains: *Levilactobacillus brevis, Lactiplantibacillus plantarum*. Samples were inoculated with cocktail of each group to a concentration of 3-4 log cfu/g and incubated at 25°C for up to 6 months. At each sampling, samples (triplicates) were removed and plated onto Yeast and Mould agar (YM) and Wallerstein Laboratory Nutrient (WLN) for yeasts, De Man–Rogosa–Sharpe agar (MRS), Nocive Brewers Bacteria Agar (NBB)for enumeration of lactic acid bacteria (LAB), and respectively. Differences among the treatments were determined using one-way ANOVA using JMP Pro version 16.1.0 (SAS Institute Inc., NC, US), with significance set at $p \leq 0.05$.
***Results*:** Samples with Kerry smoke went below detection level (1.3 log CFU/ml) at a dosage of 0.5% on Day 7 whereas the control reached spoilage levels (7 log CFU/ml) on Day 41. Enrichment was performed to validate if the strains were still viable. Enrichment confirmed the presence of strains in the seltzers. Interestingly, no significant differences ($p>0.05$) were observed between the two seltzers in terms of the performance of Kerry smoke, while alcohol levels differed from 4 to 8%.

Poster

Significance: Understanding the impact of antimicrobials and product proximates on the rate of spoilage in beverages will help product developers formulate hard seltzers with increased shelf life and quality.

P3-66 Isothermal Inactivation Kinetics of *Salmonella* Montevideo on Partially Dried Apple Cubes

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❖ ***Developing Scientist Entrant***

Introduction: The dynamic nature of drying from high to low water activity (aw) poses challenges in predicting microbial lethality. Additional data on the thermal inactivation kinetics of *Salmonella* on apple cubes can assist in predicting microbial inactivation during drying.
***Purpose*:** To investigate *Salmonella* inactivation on partially dried apple cubes with different a_w during isothermal treatment at various temperatures.
***Methods*:** Gala apple cubes (6.40mm) were pre-dried to a_w 0.60 or 0.75. *Salmonella* Montevideo was harvested from lawn culture grown on tryptic soy agar with yeast extract (TSAYE) and inoculated onto the pre-dried apple cubes (3% V/W) to achieve ~8 log CFU/g population. After ~96h re-equilibration (60 or 75% RH), inoculated apple cubes were packed into aluminum test cells under controlled RH and isothermally treated in water bath. At various time points (n=6), triplicate samples were collected and cooled in ice-water bath, and *Salmonella* was enumerated on TSAYE with ammonium iron citrate and sodium thiosulfate.
***Results*:** Following post-inoculation equilibration at 60% and 75% RH, the a_w of apple cubes was 0.61 ± 0.01 and 0.76 ± 0.01, respectively. *Salmonella* populations post-equilibration were significantly greater (p<0.05) on apple cubes at a_w 0.60 than 0.75 (8.42 ± 0.24 and 7.90 ± 0.31 log CFU/g, respectively). At 0.60 a_w, D-values were 39.27 ± 1.96, 11.21 ± 0.36, and 3.20 ± 0.20 min at 60.0, 67.5, and 75.0°C, respectively. At 0.75 a_w, D-values were 42.17 ± 1.86, 7.96 ± 0.37, and 1.50 ± 0.14 min at 52.5, 60.0, and 67.5°C, respectively. Higher (p<0.05) $D_{60°C}$ and $D_{67.5°C}$ were found on a_w 0.60 apple cubes compared to a_w 0.75 apple cubes. Similarly, z-value was significantly higher (p<0.05) for a_w 0.60 apple cubes (13.78 ± 0.50°C) than a_w 0.75 apple cubes (10.36 ± 0.36°C).
Significance: Results demonstrate that lower aw in apple cubes led to higher *Salmonella* thermal resistance (D-value) and a higher z-value.

P3-67 Factors Affecting *Salmonella* Inactivation on Apples During Hot Air Drying

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Introduction: Previous research on *Enterococcus faecium,* a surrogate for *Salmonella*, inactivation on apple cubes showed a linear relationship with aw during drying. However, data on other drying factors including temperature, airflow, and bed-depth affecting *Salmonella* inactivation is lacking.
***Purpose*:** Evaluate the effects of temperature, airflow, and bed-depth on *Salmonella* inactivation during apple drying.
***Methods*:** A six-strain *Salmonella* cocktail was harvested from lawns cultured on tryptic soy agar with 0.6% yeast extract (TSAYE) and inoculated onto Gala apple cubes (6.4 mm) at 9.41±0.21 log CFU/sample. Inoculated apple cubes were dried at low (L), medium (M), and high (H) conditions for temperature (T; 88, 104, 120°C, respectively), bed-depth (B; 5.1, 8.9, 12.7 cm, respectively), and airflow (A; 25%, 37.5%, 50%, respectively) utilizing a Box Behnken Design. *Salmonella*-inoculated apple cubes were collected at various time points (n=6) and enumerated on modified TSAYE.
***Results*:** Linear (R^2 >0.78) relationships between *Salmonella* reduction and apple cube a_w were observed for all conditions. The lowest and highest *Salmonella* reduction when reaching the same apple a_w was estimated for LTMBLA and MTLBHA. On 0.6 a_w apple cubes, estimated *Salmonella* reduction was 2.33 ± 0.42 and 4.98 ± 0.72 log CFU/sample for LTMBLA and MTLBHA, respectively. On 0.3 a_w apple cubes, estimated *Salmonella* reduction was 4.18±0.21 and 8.93 ± 0.36 log CFU/sample for LTMBLA and MTLBHA, respectively. For apple cubes dried to the same a_w, higher *Salmonella* reduction was estimated for LTMBHA than LTMBLA, and for HTMBLA than LTMBLA (p<0.05), respectively.
Significance: Higher temperatures and airflow led to similar or higher *Salmonella* inactivation in apple cubes dried to the same aw. The effect of temperature was more pronounced when using low airflow, and effect of airflow was more significant when using low temperature. Higher bed-depth delayed the inactivation but did not affect the inactivation achieved at target aw.

P3-68 Significance of Tempering Conditions on the Distribution of *E. coli* in the Milling Fractions Produced during Lab-Scale Hard Red Winter Wheat Milling

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Introduction: Contamination of wheat flour with enteric pathogens has become a major concern for the wheat milling industry. The tempering step in milling is critical to maintaining wheat flour quality and safety.
Purpose: The purpose of this study is to determine the relevance of tempering conditions such as inoculation level, tempering moisture, and tempering temperature on the *E. coli* load of wheat milling fractions.
Methods: Hard red winter (HRW) wheat grains were used for this study. The wheat grains were pre-dried and inoculated with non-pathogenic *E. coli* (ATCC 1427, 1429, 1430, and 1431) at 4 and 6 log CFU/g. Inoculated wheat grains (500 g) were tempered under different moisture (15 and 17%), temperature (25, 35, and 45° C), and time (12, and 18 h) conditions. The tempered grains were milled using lab-scale roller mill. The mill fractions were then collected, and their *E. coli* load was enumerated.
Results: The inoculation method used resulted in an inoculation level of 5.8 ± 0.2 (6-log) and 3.6 ± 0.1 log CFU/g (4-log) in the HRW wheat grains. The *E. coli* load of the grains was reduced by 0.42 to 2.65 log CFU/g, wherein greater (p < 0.05) reductions were observed at higher temperature (45° C) and inoculation levels (6-log). The *E. coli* load recovered were also higher (p < 0.05) in the non-flour fractions (bran, fine bran, and shorts), and flour-fractions (break, sizing, reduction, and straight-grade flours) when the wheat grains are tempered at higher temperatures (45° C), and inoculation level (6-log). Overall, inoculation level (9.2 to 51.49%) and temperature (19.5 to 62.6%) were the main contributors (p < 0.05) on the total variance of load reductions and *E. coli* recovered in the mill fractions.
Significance: The results from this study can be used to determine potential improvements in the tempering process to maintain wheat flour microbial safety.

P3-69 Isothermal Inactivation of *Salmonella* in Apple Slices as Affected by Water Activity

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❖ ***Developing Scientist Entrant***

Introduction: Commercially dried apples must comply with the Food Safety and Modernization Act rule for preventative controls. Because temperature and moisture are known to affect pathogen thermal inactivation, and change simultaneously, data are needed to describe those complex relationships relative to apple drying.
Purpose: The objective was to quantify the effects of temperature and water activity (a_w) on *Salmonella* inactivation in apple slices under isothermal conditions.
Method: Apples (cv. Gala) previously stored under controlled atmosphere were rinsed and sliced into 4-mm thickness. Fresh slices (0.99 a_w) were surface

inoculated with a 6-strain *Salmonella* cocktail (8.37±0.15 log CFU/g), cut into 19-mm diameter disks, and loaded into sealed aluminum test cells. To create samples at 0.2 to 0.7 a_w, slices were dried in a convection oven at 55°C up to 5 h, conditioned in humidity-controlled chambers (2-4 d), surface inoculated, cut into disks, and re-conditioned (3-5 d) to the target a_w before loading into test cells. For each of 9 temperature-a_w treatments (3x3), samples in test cells (n = 18) were heated in an isothermal water bath (60, 70, or 80°C), then removed in triplicate at 6 time points. Survivors were enumerated on a differential/non-selective medium (37°C, 48 h). Treatment effects on *Salmonella* reduction were tested via ANCOVA (α = 0.05), and D-values were estimated using a log-linear model.

Results: Overall, *Salmonella* resistance in the apple slices increased with decreasing a_w ($P < 0.05$). For example, when the a_w was 0.406±0.005, 0.715±0.001, and 0.986±0.003 (corresponding moisture contents of 12.32±0.77, 25.41±0.09, and 85.47±0.67%, respectively); the D-values at 60°C were 72.81±2.83, 16.73±0.86, 0.54±0.04 min, respectively. Depending on temperature, decreasing a_w from fresh to dried apples increased *Salmonella* resistance by up to 100 folds.

Significance: Moisture reduction increases *Salmonella* thermal resistance during apple drying, which is important for modeling thermal inactivation and process validation.

P3-70 High-Temperature Water Activity Variance among Different Proportioned Egg Powders and Its Impact on Thermal Resistance of *Salmonella* Enteritidis PT 30

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❖ ***Developing Scientist Entrant***

Introduction: Food models constructed from different food ingredients often have numerous physicochemical property differences, which limits the research on the impact of food components on high-temperature water activity.

Purpose: To investigate the variance in high-temperature water activity and its impact on the thermal resistance of Salmonella Enteritidis PT 30, through food models with different component ratios and relatively minor physicochemical differences proportioned by egg powders.

Methods: Seven model food samples were proportioned by egg powders (egg white powder, whole egg powder and egg yolk powder) and the physicochemical indexes (sample particle size, microstructure, powder flowability, etc.) were determined. The differences in high-temperature water activity were determined using the relative humidity method, net isosteric heat of sorption was calculated using the Clausius-Clapeyron equation, and the moisture sorption isotherms were modeled. Finally, the thermal resistance of *Salmonella* Enteritidis PT 30 was determined in part of the samples, and the effect of high-temperature water activity on the thermal resistance was verified by applying the above model.

Results: The results showed that the wide range of the chemical compositions of samples (e.g., fat: 0.66-56.71%; protein: 31.84-83.47%) was accompanied by limited changes in physicochemical properties compared to other model foods. The high temperature water activity and related indexes all showed regular changes with sample compositions (e.g., qst values increased gradually with fat content within 3.786 - 23470 KJ/mol). The log-linear fit of Salmonella heat resistance (D_{85} value) to high-temperature water activity ($R^2 = 0.93$) was better than that of room-temperature water activity ($R^2 = 0.78$).

Significance: This study further confirmed the influence of food components on high-temperature water activity and provided scientific evidence for the precise pasteurization of low-moisture foods.

P3-71 Non-Invasive Measurement of High-Temperature Water Activity in Proportioned Egg Powders by Raman Spectroscopy

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Introduction: High-temperature water activity (aw,T) is a crucial factor influencing bacterial thermal resistance in thermal processing, but commercial aw meters fail to measure the aw of foods at temperatures above 50□, while self-designed high-temperature aw cell still have mechanical problems.

Purpose: Our study aimed to perform a non-invasive measurement of $a_{w,T}$ with broad range of $a_{w,25℃}$ (0.11 - 0.94) and diverse food components (e.g. fat and protein ratio) using Raman spectroscopy.

Methods: Three forms of egg powders (egg white powder, egg yolk powder, and whole egg powder) and their mixtures (35 in total) were selected as model foods. Their $a_{w,T}$ at 25 - 75℃ were measured by high-temperature a_w cells designed by Washington State University. Raman spectra of egg powders, filled into self-designed quartz glass cells, were acquired at 25 - 75℃, using 785 nm excitation at 175 - 4150 cm^{-1}. Chemometrics methods and intelligent algorithms were used to fit and model the measured data.

Results: The result shows that the coefficient of correlation ($R^2 = 0.888$) is higher for the selected Raman shifts in the ranges 800 - 1800 cm^{-1} and 2800 - 3100 cm^{-1} than for the full wavelength band ($R^2 = 0.764$). By comparing multiple pre-processing methods and regression algorithms, SNV-SVR (linear kernel function) performs well in building the water activity quantitative model, with the R^2 of 0.989 and RMSEV of 0.031. By utilizing feature variables filtering and low-field NMR techniques, we further discuss possible mechanism for predicting water activity by Raman spectroscopy.

Significance: Our study provided a new insight on using Raman spectroscopy to model the aw of low-water activity foods at elevated temperature. Raman spectroscopy appears to be a non-invasive technique that provides a novel perspective for accurate and real-time measurement of foods during the thermal pasteurization.

P3-72 A Surrogate to Challenge and Validate Cleaning and Sanitation of Low-Moisture Food Persistent Bacterial Populations (LMF PBP)

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Introduction: Low-moisture food persistent bacterial populations (LMF PBPs) may be responsible for pathogen harborage in LMF processing environments, resulting in outbreaks and recalls. Cleaning and sanitation strategies must be tailored to different equipment and environments, and in-plant validations would assist with evaluating the efficacy of sanitation protocols that eliminate LMF PBPs and harborage.

Purpose: This study explored surrogates' representative of a pathogen LMF PBP, providing tools for challenging or validating cleaning and sanitation practices eliminate LMF PBPs from surfaces.

Methods: Three surrogates (*E. coli* MP 26, *Enterococcus faecium*, *Pediococcus acidilactici*) were evaluated for similarity to a *Salmonella* LMF PBP in nonfat dried milk (NFDM) formed on stainless steel coupons stored at 30% or 70% relative humidity (RH) and 25°C. Coupons were prepared with a previously established method to form an LMF PBP (0.5 g of NFDM, 0.1 mL organism). The survival of each microorganism was determined by enumeration over 14 days. Bacterial survival and D-values were calculated and analyzed with ANOVA and Tukey's HSD to determine significant differences.

Results: The D-values of all microorganisms increased with reducing RH. At 30% RH in NFDM, D-values of *Salmonella, E. faecium*, and *P. acidilactici* were 7.1, 14, and 46.8 days, respectively; which were higher than liquid cultures dried to the coupon without a food matrix (3.7, 11.6, and 43.7 days).At 70% RH, these values of LMF PBPs in NFDM were significantly lower at 5, 11.1, and 28.7 days in NFDM, and 2.5, 5.7, and 27.3 days as dried cultures without a matrix ($p<0.05$).

Significance: E. faecium or P. acidilactici LMF PBPs are similar but hardier than Salmonella LMF PBPs and could be used in processing environments for challenging and validating sanitation protocols, addressing limitations of current studies that used biofilms or dried cultures on surfaces.

P3-73 Impact of Inoculum Growth Method on Survival of *Salmonella* and Shiga-Toxin Producing *Escherichia coli* (STEC) during Wheat Tempering

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❖ ***Developing Scientist Entrant***

Introduction: Previous research has highlighted that inoculum growth method impacts pathogen survival during desiccation, and thermal tolerance on low-moisture foods. Using an antimicrobial solution for wheat tempering is a cost-effective flour safety intervention; however, the lack of information on pathogen responses when prepared with different inoculum methods, and differences in experimental design make it difficult to compare results across studies.

Purpose: To determine statistically significant parameters for tempering experiments and to quantify pathogen changes due to various tempering treatments.

Methods: Five-strain cocktails of *Salmonella* (serovars Enteritidis PT30, Agona, Mbandaka, Tennessee, Montevideo) and STEC (serotypes O157:NM, O157:H7, O157:H7, O26:H11, O121:H19) were prepared using five inoculum growth methods (broth, lawn-aerobic, lawn-anaerobic, acid-adapted, and low inoculum level). Inoculated wheat grain was tempered for 18 hours using 4 different treatments including the water control, 800 ppm chlorine, 5% lactic acid + 26.6% NaCl (LA+NaCl), and 5% sodium bisulfate (SBS). Tempering was performed 1 day after inoculation with no water activity (a_w) equilibration, day 2 after a_w equilibration, and day 7, 28, and 84 (post-a_w equilibration). The results were analyzed using CART model.

Results: Bacterial species did not have a significant impact (p>0.05) on pathogen survival during tempering. Water, chlorine, SBS, and LA+NaCl led to average reduction of 0.52, 1.04, 1.43, and 1.96 log CFU/g in the bacterial population respectively, which were significantly different (p<0.05). The inoculum growth method had significant (p<0.05) impact on bacterial survival. In general, the reduction of pathogens on grain grown in broth = lawn-anaerobic > low inoculum ≥ lawn-aerobic ≥ acid-adapted inoculum. Pathogen adaptation time had significant (p<0.05) impact on pathogen reduction when tempered with water and LA+NaCl.

Significance: The effectiveness of the tempering treatment was impacted by the inoculum growth method; therefore, a consistent inoculum growth method should be promoted for comparison across research studies.

P3-74 Assessment of *Enterococcus faecium* ATCC 8459 as a Surrogate for *Salmonella* in Baked Cookie Products

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Introduction: *Enterococcus faecium* ATCC 8459 is a commonly used nonpathogenic surrogate bacteria for the validation of thermal processes for *Salmonella*. However, limited studies evaluate surrogate microorganism appropriateness in industrial-scale, baked cookie products.

Purpose: The study aims to assess *E. faecium* ATCC 8459 as a potential surrogate bacterium in low-water activity and high-fat products comparing thermal resistance and sensitivity (D-values and z-values) and population reduction to a 3-serovar *Salmonella* cocktail (Newport, Typhimurium, and Senftenberg).

Methods: Strains were cultured and concentrated using multiple centrifugations to inoculate flour (~8 log CFU/g). An inoculated low water-activity dough (0.650 ± 0.02) and a high-fat content dough (24.34% ± 1.19%) were formed into 25-gram cookies (~7 log CFU/g), vacuum-sealed, placed in a water bath at 68°C, 71°C, and 74°C with 7 duplicate time pulls and recovered for population counts. Each dough was replicated three times per temperature (n = 126). Statistical significance was determined using a two-factor ANOVA.

Results: D-values of low water activity *E. faecium* and *Salmonella* dough were 50.90 and 43.83 at 68°C, 44.42 and 31.65 at 71°C, and 30.49 and 23.59 at 74°C, respectively, with a z-value of 27.00 and 21.05. D-values of high-fat content *E. faecium* and *Salmonella* dough were 126.13 and 45.32 at 68°C, 62.68 and 19.84 at 71°C, 50.00 and 19.29 at 74°C, respectively, with z-values of 13.69 and 14.91. Population reduction recoveries found *Salmonella* had statistically significant higher population reductions for all time points and temperatures except time 40 at 68°C in high-fat content dough and three-time points in low water activity dough (p < 0.05). No significant differences were found for other time points.

Significance: The results provide support for *Enterococcus faecium* as an appropriate surrogate for the thermal inactivation of *Salmonella* in baked products incapable of other validation methods.

P3-75 Effect of Fermented Wheat Flour on the Shelf Life of White Bread

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Introduction: Microbial spoilage of bread has significant challenges leading to food waste and economic losses. Clean label preservation solutions emerge as a viable solution to mitigate mold formation and extend shelf life.

Purpose: Evaluate the impact on the shelf life by incorporating fermented wheat flour into white bread.

Methods: Standard white bread loaves were prepared with three different formulations: 0.3% lactic acid (control), fermented wheat flour (1.5% UpGrade™ WS) combined with 0.3% lactic acid (T1), and 0.5 % calcium propionate with 0.2% lactic acid (T2). The study involved inoculation to evaluate the behavior of common mold species typically found in bakery production sites: *Aspergillus niger*, *Penicillium roqueforti,* and *Cladosporium cladosporioides*. The bread samples were securely sealed in plastic bags and stored at 20°C. Daily observations were made on the bread surface crust to monitor the growth of mold. Differences among the treatments were determined using one-way ANOVA at $p<0.05$.

Results: For *C. cladosporioides*, T1 and T2 showed significant ($p<0.05$) increase in days to mold >29 days compared to control (7 days). Similar result pattern was observed for T1 with *A. niger* (days to mold >29 days) whereas for T2 it molded at day 19 compared to control which molded at day 4. The performance of fermented wheat flour (T1) against *P. roqueforti*, identified as the most resistant strain, was significantly better (days to mold 8 days; $p<0.05$) than calcium propionate (T2) and control, that molded at day 6 and 4, respectively.

Significance: Fermented wheat flour is shown to be a suitable clean label alternative to calcium-propionate in extending the shelf life of white bread.

P3-76 The Antimicrobial Effects of an in vitro Spore Production and Co-Inoculation Assessment of Bacillus Strains on Pre-Packed Flour Tortillas Using Targeted Directional Microwave Technology

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Introduction: The ropiness in flour tortillas is considered a re-emerging challenge, necessitating further research to develop innovative methods, including hygienic techniques, to prevent the occurrence of *Bacillus* strains' spores and mitigate their bacterial rope-forming potential.

Purpose: (1) To evaluate the effectiveness of MicroZap technology treatments in reducing viable spores of *Bacillus* strains on flour tortillas and managing bacterial contamination without compromising the product shelf-life.

Methods: For each trial, twenty-five flour tortilla samples were obtained from a supermarket bakery. Treatments were applied as follows: The treatment levels were applied as follows: TI: after treatments were applied, the highest LR achieved was 2.13 Log10 CFU/g and a minimum of 1.01 Log_{10} CFU/g.s, Tr2=

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(10ct) 212.6 kw*s, and Tr3= (10ct) 243.0 kw*s; TII: Tr1= (10ct) 265.8 kw*s, Tr2= (10ct) 267.0 kw*s, and Tr3= (20ct) 386.5 kw*s. *Bacillus* spores were produced in a formulated DSM broth, and a confocal 3D Imaging Microscope System was used for spores' visualization. Bacillus was enumerated post-treatment using APC Petrifilm with statistical analysis performed in GraphPad Prism 10 for Mac Version 10.2.2.

Results: The inoculated control samples for trials I and II were 6.66 ± 0.08 and 8.0 ± 0.05 log CFU/g, respectively. In trial I, after treatments were applied, the highest LR achieved was 1.53 log CFU/g and a minimum of 0.41 log CFU/g. In trial II, after treatments were applied, the highest LR was 2.39 log CFU/g and a minimum of 1.10 log CFU/g. Samples inoculated had higher energy levels in Trial I (Tr1) and Trial II (Tr2 and Tr3) (p-value 0.05) compared to the other treatments. The reductions for treatments vary at incremental power levels and package counts.

Significance: This research illustrates a collaborative approach between industry and academia in devising a delivery strategy. The focus is integrating methods for evaluating the potential of bacterial rope formation using actual food-matrix data, aiming to tackle practical challenges encountered by the food industry.

P3-77 Inactivation of *Salmonella enterica*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* by Searing in Sub-Optimal Sous-Vide Cooked Beef Steaks

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❖ ***Developing Scientist Entrant***

Introduction: Sous-vide cooking is popular in restaurants and the catering industry due to its benefits, including uniform cooking, preventing recontamination, and precise control over doneness. Our previous studies assessed cooking and storage parameters for both intact and non-intact steaks. Quantifying pathogen inactivation during searing after refrigeration is essential for a more comprehensive food safety assessment of the entire process.

Purpose: This study evaluated pathogen inactivation during searing of sub-optimally sous-vide cooked steaks using two different time-temperature combinations.

Methods: Eye of round steaks (1 inch thick, 100±3g) were cut and individually surface inoculated with five strains of *Salmonella enterica*, three strains of *E. coli* O157:H7, and three strains of *Listeria monocytogenes*. To create non-intact steaks, a jaccard tenderizer was used. After pathogen attachment (30 mins) and vacuum packaging, steaks were cooked at 57.5℃ for 16 mins, resulting in a residual microbial load of 5±1 log CFU/g. After crash chilling and refrigeration, samples were seared at 177℃ for 2 mins or 232℃ for 1.5 mins on each side, followed by enumeration. Data were analyzed using a mixed linear model in SAS (Version 9.4).

Results: No difference (p>0.05) was observed between cooking at 177°C for 2 minutes and 232°C for 1.5 minutes. Searing reduced *Salmonella* counts from 4.77±0.31 log CFU/g to 3.19±0.31 log CFU/g in intact steaks, but not in tenderized steaks. *E. coli* reduced from 4.70±0.44 log CFU/g to 2.62±0.44 log CFU/g in intact steaks and from 5.63±0.44 log CFU/g to 4.85±0.16 log CFU/g in tenderized steaks. Similarly, *Listeria* reduced from 4.56±0.16 log CFU/g to 2.60±0.16 log CFU/g in intact steaks and from 5.02±0.16 log CFU/g to 3.54±0.16 log CFU/g in tenderized steaks by searing.

Significance: Searing provides additional inactivation of pathogens in sub-optimally sous vide cooked steaks, but tenderization limits the added benefit.

P3-78 Examining Interventions for Dry-aged Steak Crusts Inoculated with Salmonella Heidelberg, Escherichia coli O157:H7, and Listeria monocytogenes 4b

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Introduction: Dry aging, a culinary technique involving aging subprimal steaks in a controlled dry environment for 28-55 days, enhances unique flavors, but may also lead to the growth of pathogenic bacteria and fungi. Typically, the resulting crust is trimmed and discarded; however, interventions to reduce populations could prevent this waste.

Purpose*:* Evaluate the effectiveness of interventions in reducing populations of *Salmonella, E. coli,* and *L. monocytogenes* from dry-aged beef crusts.

Methods*:* Steak crusts were spot inoculated with a cocktail of rifampicin-resistant pathogens (*S. Heidelberg*, *E. coli* O15:H7, *L. monocytogenes* 4b) at 7.0 log CFU/ml. Pre- and post-treatment crusts for six treatments and negative and positive control were sampled in triplicate (N=24). The treatments were hot-air drying (6 h, 60°C), *sous-vide* (2 h, 60°C), ambient water spray (1 min, 15°C), hot water spray (1 min, 50°C), 2% lactic acid spray (1 min), and UV-C light (30 min, 100-280 nm). Two cores from each crust (9.81 cm^2) were excised, diluted, and spiral-plated onto Modified Oxford Agar and Sorbitol MacConkey Agar (with 200 ug/ml rifampicin). Log reductions were calculated based on initial inoculation levels.

Results*:* Among the six treatment methods, hot air drying exhibited the highest efficacy with a log reduction of ≥5 log CFU/cm^2 for *E. coli* and *S. Heidelberg* (5.7 and 6.3 log CFU/cm^2, respectively), but only a 2.2-log CFU/cm^2 reduction for *L. monocytogenes*. The remaining treatments had lower efficacy, with reductions ranging from 0.1-4.9 log CFU/cm^2 for the pathogens.

Significance: These findings underscore the effectiveness of hot-air drying as a method to achieve substantial log reductions in E. coli and S. Heidelberg, providing valuable insights for interventions to mitigate pathogen growth in dry-aged beef crusts and reduce waste.

P3-79 Cross-Contamination of Multi-Species Biofilms Formed on Chicken by *Salmonella* Enteritidis, *Campylobacter jejuni,* and *Clostridium perfringens*: Aerobic, Microaerobic, and Anaerobic Conditions

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Introduction: Investigation of contamination and cross-contamination related to the formation of multi-species biofilms on both food surfaces and food contact surfaces is crucial for ensuring food safety.

Purpose: This study was performed to investigate the cross-contamination dynamics of biofilms preformed on stainless steel (SS) coupons on chicken skin and chicken meat by modeling surface-to-food transfer by contact and surface-to-food transfer in fluids.

Methods: The formation of mono-, dual-, and multi-species biofilms of *Salmonella* Enteritidis isolated from poultry meat, *Campylobacter jejuni* and *Clostridium perfringens* in various environment factors including aerobic, microaerobic, and anaerobic conditions were evaluated. Crystal violet (CV) assay and extracellular polymeric substances (EPS) quantification were performed to evaluate the biofilm forming ability. In addition, bacterial distribution and cross-contamination were analyzed to investigate cross-contamination potential of biofilms on chicken skin and chicken meat.

Result: The OD_{600nm} value of CV assay revealed that a dual-species biofilm of *Salmonella* Enteritidis with *Campylobacter jejuni* (3.009 ± 0.436) and a multi-species biofilm of *S.* Enteritidis with *C. jejuni* and *Clostridium perfringens* (3.317 ± 0.505) cultivated for 72 h under aerobic conditions exhibited significantly higher biofilm-forming abilities than the other biofilm types (P<0.001). Consistent with the CV assay findings, the multi-species biofilm exhibited significantly higher relative fluorescence units of extracellular DNA (32.968 ± 3.797) and protein levels (29.817 ± 4.629 μg/mL) than the other biofilm types for 72 h under aerobic conditions. Furthermore, viable counts of dual- or multi-species biofilms formed by *S.* Enteritidis with *C. jejuni* or *C. perfringens* and all strains showed 7.291 ± 0.189, 7.169 ± 0.191, and 7.105 ± 0.259 CFU/cm^2, respectively (P < 0.001). In addition, cross-contamination models revealed that the pressing method induced greater cross-contamination across biofilms irrespective of the food sample or biofilm characteristics.

Significance: Surface-to-food contact played a more influential role in cross-contamination than surface-to-food interactions in fluids.

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P3-80 Shelf-Life Extension of Poultry Leg Quarters Treated with a Short-Duration Dip (15-s) of Sodium Acid Sulfate

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Introduction: Shelf-life extension efforts using antimicrobial dips and sprays in poultry abattoirs are ongoing. Despite these efforts, poultry shelf-life is short compared to other meat products, 7-10-d. Thus, novel antimicrobial solutions to extend raw poultry shelf-life are needed.

Purpose: Evaluate effects of sodium acid sulfate (SAS, Jones-Hamilton Co., Walbridge, OH), commercial standard, peracetic acid (PAA), and their combination as a poultry 15-sec antimicrobial dip on chicken leg quarter microbiological shelf-life characteristics.

Methods: Skin-on, bone-in leg quarters (N=300) obtained from antimicrobial-free carcasses were not treated (NT) or treated for 15-s in either tap water (TW), SAS (3%), PAA (500 ppm), or combination of SAS+PAA. Leg quarters were rested (2-min), packaged (tray with film overlay), and sampled on d0, 4, 7, 14, 21, and 28 of shelf-life for total mesophiles (APC), *Enterobacteriaceae* (EB), lactic acid bacteria (LAB), *Pseudomonas*, and yeast and molds (YM). Microbial count data (Log CFU/g) were analyzed as a GLM in R-Studio with pairwise differences determined using Tukey's (P≤0.05).

Results: No difference between APC of SAS, PAA, and SAS+PAA-treated legs on d28 (5.78, 5.56, 6.56 log CFU/g). EB was lower among SAS and SAS+PAA-treated leg quarters (3.61, 3.99 log CFU/g) than PAA-treated legs (5.91 log CFU/g) on d28. LAB of SAS+PAA-treated legs was lower than PAA-treated and controls (2.81, 4.27, 5.19, 5.29 log CFU/g) on d14. On d21, LAB was less among SAS-treated than PAA and SAS+PAA-treated legs (2.84, 5.08, 4.19 Log CFU/g). Synergistic effect of SAS+PAA, maintaining 1-1.5 log CFU/g lower of *Pseudomonas* from d7 to 28 compared to those treated with SAS and PAA. Similarly, SAS+PAA controlled YM on d7 and 14 compared to other treatments (1-2 log CFU/g lower).

Significance: SAS+PAA synergistically controlled *Pseudomonas* and YM, whereas SAS controlled EB and LAB more effectively. Overall, SAS and SAS+PAA use extended poultry leg quarter shelf-life to 21-d.

P3-81 Biomapping Microbiological Indicators at Different Stages in the Food Service Chicken Tenders Distribution Value Chain under Different Cold Storage Conditions

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Introduction: Poultry industry has a substantial positive impact on the world economy and food supply. Poultry is perishable if the cold distribution chain is ineffective, and temperature abused poultry products can spoil and contribute unnecessarily to food waste. Changes in indicator and spoilage organisms in the cold distribution value chain need to be understood to identify intervention strategies and model bacterial growth to prevent spoilage.

Objective: Determine the dynamics of microbial indicators and spoilage organisms in food service chicken tenders under three different simulated cold distribution value chains: refrigeration, conventional-freezing and blast-freezing conditions.

Methods: 11 sampling points in the cold distribution value-chain of chicken tender was conducted. Three storage treatments were applied: fridge (4°C), freezer (-18°C), and blast freezer (-40°C) storage. 5 repetitions were conducted. Tempo® protocols were performed to quantify aerobic total counts, Enterobacteriaceae, and psychrotrophs.

Results: All indicators increased during the refrigeration cold storage simulation, although stability was noted at days 11, 14, 17 and 20 for AC and PSY, a statistically significant difference ($p<0.05$) was found in the growth of PSY. In the freezer and blast-freezer simulations, AC and EB populations remained at 2.644 log CFU/g, with the exception of PSY, which a four-log increase. PSY counts were highest (5.68 log CFU/g) in all storage types, although at freezer and blast-freezer temperatures, their numbers decreased before stabilizing between 1.4-1.8 log CFU/g. In contrast, APC (2.7 log CFU/g) and EB (1.49 log CFU/g) showed the lowest counts in all storage types.

Significance: It is essential to understand the dynamics of microbial indicators and spoilage organisms in the cold chain distribution. This study provides data on the dynamics of microbial loads at different stages of the distribution chain of chicken tenders destined to food service channels. Data can be used to establish statistical process control parameters for food quality management.

P3-82 Development of a Data Visualization Tool for Determining Minimum Inhibitory Concentration for Antimicrobial Active Values and Facilitate Ingredient Selection

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Introduction: Determination of minimum inhibitory concentration (MIC) values is a crucial operation in the food safety industry for the adequate selection of antimicrobial formulations. With the vast amount of data generated by the industry, and significant number of antimicrobial ingredients available, the development of a tool to present and visualize MIC in a rapid, user-friendly, and precise manner has become a necessity.

Objective: Develop a user friendly and effective tool for visualizing a large volume of MIC values for ingredient selection decision-making.

Methods: A series of excel files (n=156) containing growth curve data developed to evaluate the response of a specific microorganism to various concentrations of an antimicrobial compound using a Bioscreen C equipment were analyzed. The generated data underwent analysis to calculate lag phase and maximum growth rate values for each microorganism and active compound. MIC values were determined using a Hill equation. The dataset encompasses MIC calculations that evaluate the combination of different intrinsic factors, including pH, Aw, microorganism, antimicrobial compound, MIC type, and data quality.

Results: A visualization tool was developed using the Shiny package of the R software. This tool enables the selection of data (1638 MIC values) through filter options, allowing users to filter data based on various factors such as pH, Aw, microorganism, compound, MIC type, and data quality. The tool consolidates all the data into a single screen, presenting it in the form of a bubble map where each bubble represents a unique MIC value. The user can then rapidly identify the most effective antimicrobial and factor combination for microbial control.

Significance: A visualization tool for MIC data analysis enhances the industry's decision-making capacity during the product formulation stage of food products. This tool streamlines the work for scientists, accelerating developments, by simplifying the process of finding product specific and accurate information for microbial control and shelf-life extension.

P3-83 Evaluating Potassium Vinegar Systems and Vinegar Fermentate Blend for Spoilage Control and Sensory Perception in Fresh Chicken Tenders

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Introduction: Traditionally, vinegar has been used as a marinade solution due to its ability to impart improved flavor and tenderness in poultry. Understanding the antimicrobial impact of vinegar systems can help processors extend shelf life of fresh poultry.
Purpose: To validate the antimicrobial effect of liquid vinegar systems; sodium (IsoAge 220L, 230L, 240L) and potassium-based vinegar (300L and 325L) as marinades for controlling growth of spoilage microorganisms in marinated chicken tenderloins.
Methods: Fresh chicken tenders were treated with different IsoAge (220/230/240/300/325L) at 1.0 and 2.0 % concentration. Samples were subjected to vacuum tumbling-immersion marination in chilled water, salt, and IsoAge at 8-10 rpm for 30 min at 4˚C. Brine solution (without preservative) served as control. Treated samples (~100 g) were vacuum-packed, stored at 4°C for up to 30 days. On each sampling day, duplicate samples were enumerated for lactic acid bacteria (LAB) and aerobic plate count (APC). Spoilage threshold was set at 6 log CFU/g. Statistical analysis was performed in JMP Pro version 16.1.0 (SAS Institute Inc., NC, US), with significance set at $p<0.05$.
Results: IsoAge 220, 230, 240, 300L and 325 L at 2.00% exhibited superior efficacy in extending the shelf-life of chicken tenders as compared to 1.00% and control ($p<0.05$). Specifically, chicken IsoAge 220 and 230 L at 2.00% achieved a 27-day shelf-life, while IsoAge 240, 300L and 325 L achieved 23-day shelf-life ($p<0.05$). IsoAge 220 and 230 L also significantly delayed growth of LAB (days to spoilage 27 and 22 days; $p<0.05$), followed by IsoAge 240, 300L and 325 L (days to spoilage 17 days) surpassing control (brine solution), which spoiled by day 7 ($p<0.05$).
Significance: Sodium and Potassium vinegar systems can significantly extend the shelf-life of marinated poultry, providing processors with means to improve microbial stability and poultry meat quality.

P3-84 Inhibition of Lactic Acid Bacteria in High-Moisture Fresh Pet Food Using Vinegar and Fermentate System

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Introduction: Fresh pet food, with its higher water activity (aw) and moisture content, faces challenge with shorter shelf-life and increased susceptibility to microbial growth. To assess the growing trend of humanizing pet food, manufacturers are seeking sustainable, clean-label solutions to enhance microbiological safety of fresh pet food.
Purpose: To assess the inhibitory effect of vinegar/peptide ferment against Lactic Acid Bacteria (LAB) isolated from high-moisture pet food through *in-vitro* screening.
Methods: Broth screening was conducted using clean-label antimicrobials: 1.65% buffered vinegar/smoke and 2.5% vinegar/peptide ferment (EverWild-20L) in De Man, Rogosa and Sharpe (MRS) broth (pH 6.0). Samples were aliquoted into 100-well honeycomb plates and inoculated with LAB (*Lactobacillus cellobiosus*, *Lactobacillus sakei*, *Enterococcus faecalis, Enterococcus faecium,* and *Lactobacillus curvatus*) that were previously isolated from chicken-based pet foods. The plates were then placed in the Bioscreen-C at 37°C and optical density was measured at 600 nm. Growth curves were generated, and lag time and maximum growth rate were modelled using a Gompertz function.
Results: *In-vitro* testing indicated 2.5% EverWild-20L was effective against LAB. Particularly, 1.65% buffered vinegar/smoke and 2.5% vinegar/peptide ferment decreased μmax of *L. cellobiosus* by 1.71 and 6.32 h^{-1} respectively as compared to blank. 1.65% Buffered vinegar/smoke completely inhibited growth of *L. sakei,* whereas 2.5% vinegar/peptide ferment decreased growth by 4.26 h^{-1}. 2.5% vinegar/peptide ferment completely inhibited the growth of *E. faecalis, E. faecium,* and *L. curvatus.*
Significance: Vinegar/peptide ferment (EverWild 20L) exhibited significantly higher efficacy against target microorganisms, suggesting it's potential to prevent spoilage of high-moisture pet foods.

P3-85 Shelf-Life Enhancement of Fresh Ground Poultry Using Natural Dried Vinegar and Rosemary Extract Preservation System

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Introduction: Rising concerns over the use of artificial preservatives has spurred greater interest in natural preservatives to prolong the shelf-life of ground chicken.
Purpose: To validate the inhibition of spoilage microorganisms in fresh ground chicken treated with natural dried vinegar alone or in combination with rosemary extract (RE)
Methods: Different treatments of fresh ground chicken thighs were prepared: control (no preservation system), 0.75% dried vinegar (IsoAge DV100) alone or in combination RE at a concentration of 0.0033%, 0.0067%, 0.0102%, 0.0132%, and 0.0170%. Treated samples were packed and stored under aerobic conditions at 4°C for up to 25 days. At each sampling, samples were plated in duplicates onto Plate Count and Violet Red Bile (VRB) agar for enumeration of aerobic plate count (APC) and Enterobacteriaceae (EB), respectively. Spoilage threshold was considered as 6 log CFU/g. Data generated were used for primary modelling using modified Gompertz equation to calculate maximum growth rate (μmax; log/day) and days to reach spoilage threshold for each treatment. Treatment performance was compared using one-way ANOVA. Statistical analysis and model building was performed in JMP Pro version 15.1.0 with significance set at $P<0.05$.
Results*:* Overall, the growth of APC and EB in ground chicken was effectively controlled by 0.75% dried vinegar in combination with RE (0.0102% or greater) in combination with 0.75% dried vinegar. Particularly for APC and EB, the combination treatment, 0.75% dried vinegar + RE 0.0102% or greater enhanced the shelf-life of ground as compared to the control (no preservative) which reached spoilage threshold (6 logs CFU/g) by 6 and 8 days, respectively ($p<0.05$).
Significance: The results of this study demonstrate the enhanced efficacy of dried vinegar in combination with rosemary extract to control the growth of spoilage microorganisms while substantially enhancing the shelf-life of ground chicken.

P3-86 Effectiveness of Essential Oil Vapors to Extend the Shelf Life of Kai Lan (*Brassica oleracea* var. *alboglabra*)

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Introduction: Essential oil (EO) vapors containing abundant active components are promising candidates for food preservation. *Brassica* leafy vegetables such as Kai Lan (*Brassica oleracea* var. *alboglabra*) widely consumed in Asia, are nutritious but have a short shelf-life.
Purpose: This work was to investigate the impacts of EO vapors on the microbial quality and appearance of Kai Lan during storage at two temperatures, aiming to extend its shelf-life for better commercial benefits.
Methods: Five microbial cultures including both bacteria and molds, typically causing the spoilage of leafy vegetables, were challenged for the EO screening. The vapor contact assays of cinnamon, thyme, oregano, clove, and basil EOs were conducted by the inverted petri plate method. Changes in microbial communities after the exposure to selected oregano EO vapor at 25 ℃ and 7 ℃ were examined by total aerobic counts, 16S V3-V4 amplicon, and 18S V4 amplicon sequencing. The impacts on the appearance of Kai Lan were demonstrated by photographing and measurements of chlorophyll and

carotenoids.

Results: Cinnamon and oregano EO vapors showed the highest antimicrobial effects against the spoilage-causing pure cultures, while the oregano EO vapor (as low as 10 µL in a 1.8 L container) was more effective in the chlorophyll preservation. The impacts of oregano EO vapor on the microbial loads and color of Kai Lan followed a dose-dependent manner. The high-throughput sequencing revealed that the EO vapor drastically altered the microbial loads and the microbial composition on Kai Lan leaves.

Significance: This work demonstrates the potential of using EO vapors to extend the shelf-life of Kai Lan with better sensory and microbial quality.

P3-87 Minimum Inhibitory Concentrations of Propionic Acid, Benzoic Acid, and Sorbic Acid in Fruits

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❖ ***Developing Scientist Entrant***

Introduction: Preservatives are used to control the growth of microorganisms in foods. The minimum inhibitory concentrations (MICs) of the preservatives in food vary because of pH, nutritional contents, and other factors in each food.

Purpose: The objective of this study was to determine the MICs of propionic acid (PA), benzoic acid (BA), and sorbic acid (SA) in fruits.

Methods: Twenty-five grams of each fruit (avocado, papaya, banana, coconut, durian, lychee, jujube, fig, and persimmon) were inoculated with a mixture of foodborne pathogenic and spoilage bacteria, such as *Campylobacter coli*, *Campylobacter jejuni*, *Erwinia carotovora* subsp. *carotovora, Moraxella catarrhalis*, and *Micrococcus luteus* at 3 - 4 log CFU/g. The inoculated samples were spiked with PA, BA, and SA at 0 ppm (control), 100, 500, 1,000, and 1,500 (1,200 ppm for SA). The inoculated samples were stored at 25℃ until the bacterial cell counts in the control reached 6 log CFU/g. The bacterial cell counts were enumerated on the Petrifilm™ aerobic count plate. The concentrations that had no increase in the cell counts between after inoculation and the end of the storage were determined as the MICs.

Results: The MICs were 500 ppm in papaya, coconut, and persimmon, regardless of preservatives. In avocado, the MICs were 1,000 (PA), 500 (BA), and 500 (SA) ppm. The MICs were 500 (PA), 100 (BA), and 100 (SA) ppm in banana, and the MICs were 1,000 (PA), 1,000 (BA), and 500 (SA) ppm in fig. The MICs were 500 (PA), 20 (BA), and 100 (SA) in jujube. In lychee, the MICs were 1,500 (PA) and 1,500 (SA), whereas SA did not show inhibition effects at 1,200 ppm.

Significance: In durian, PA, BA, and SA did not show inhibition effects. This result indicates that the MICs of the examined preservatives vary in fruits. It might be related to pH of food and natural antimicrobials in fruits.

P3-88 Antimicrobial Photodynamic Inactivation of *Alicyclobacillus acidoterrestris* Spores on Orange Surface Using an Original Device

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Introduction: The presence of *Alicyclobacillus acidoterrestris* spores may cause fruit juice spoilage main due to guaiacol production since pasteurization is not able to inactivate the spores.

Purpose: The investigation explores the efficacy of antimicrobial photodynamic treatment (APDT) using new methylene blue N (NMBN), curcumin (CURC), and 8-methoxypsoralen (8-MOP) as photosensitizers against *A. acidoterrestris* spores.

Methods: The spore suspension of *A. acidoterrestris* (DSM 2498) was prepared in Roux bottles containing YSG agar (pH 3.7), supplemented with manganese sulfate at 10 ppm. Stock solutions of the photosensitizers were prepared with water (NMBN) or DMSO (8-MOP and CURC). Light exposure involved three types of light sources: red light-emitting diode (LED), blue LED, and solar radiation. Subsequently, a minimal inhibitory concentration (MIC) assay, performed in triplicate, assessed the optimal conditions (light exposure time and photosensitizer concentration) for APDT before application to oranges artificially inoculated with spores of *A. acidoterrestris* using an original device. Light and dark controls were included to assess the isolated effects of light and photosensitizers, respectively.

Results: The results from MIC assay determined that the following experiments on the surface of oranges would be conducted with concentrations of 125 µM for NMBN and 160 µM for both CURC and 8-MOP. APDT in the presence of NMBN and red LED reduced spore count by 1.06 logs after 180 min ($p < 0.05$). The combination of CURC and blue LED reduced spore count by 0.88 logs after 180 min ($p < 0.05$). APDT with 8-MOP was able to reduce up to 0.85 and 1.49 log ($p < 0.05$) spores during a 120 min of solar radiation in the morning and the afternoon, respectively. Thus, considering the low contamination in oranges, 8-MOP and afternoon solar radiation was the most effective APDT treatment.

Significance: Results obtained in this study can contribute to future APDT developments in different conditions and matrices, including field applications.

P3-89 Development of a Sustainable Antifungal System Using a Synergistic Treatment of Aqueous Olive Pomace Extract (OPE) and Sunlight to Control *Alternaria* Infection on Tomato Plants

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❖ ***Developing Scientist Entrant***

Introduction: Agricultural byproducts have the potential to develop sustainable and low-cost sources of photoactive antifungal agents that can exert strong synergistic antifungal activity upon exposure to sunlight.

Purpose: This study was conducted to evaluate the antifungal potential of the crude olive pomace extract (OPE) with sunlight to inhibit *Alternaria* infection on tomato skins and leaves.

Method: The OPE was obtained from dried olive pomace using water-based, ultrasound-assisted extraction (UAE; 100W, 42 kHz, 20 min). The minimal inhibitory concentration (MIC) of OPE against *Alternaria alternata* was determined using a germination tube assay. The synergistic antifungal activity of OPE with sunlight was evaluated on tomato skins and leaves using a plate count assay. An *in vivo* test was conducted to evaluate the synergistic activities of OPE and sunlight in inhibiting the *A. alternata* infection on tomato leaves. The spread of the infection on tomato leaves was monitored during 10 days of storage after the combined treatment.

Results: The OPE obtained using water-based UAE exerted strong antifungal potential against *A. alternata* (MIC: 2.0 mg GAE/mL). The combined treatment of OPE and sunlight exerted strong synergisms on tomato skins and leaves and achieved ca. 2.73 and 2.21 log reductions of *A. alternata* conidia within 30 min, respectively. The combined treatment of OPE and sunlight exhibited strong synergism in inhibiting the spread of *A. alternata* infection on tomato leaves. The infected tomato leaves exposed to the combined treatment showed about a 2.4-fold increase in the size of the infection area during 10 days of storage, whereas those treated solely with DW, OPE, or sunlight showed about 18.0, 20.5, or 7.9-fold increases after the storage.

Significance: These findings will illustrate the novel application of ag-by products to develop sustainable antifungal agents and synergistic enhancement in their activity upon exposure to sunlight to control plant fungal pathogens.

Poster

P3-90 Development of a Food-Grade, Bio-Based Antimicrobial Coating for Low-Moisture Food (LMF)-Handling Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Microbial cross-contamination of low-moisture food (LMF), such as wheat flour, with bacterial pathogens poses a serious safety and economic concern.

Purpose: This study was conducted to develop a food-grade, bio-based antimicrobial coating that can be readily applied to food-handling surfaces and prevent the surface-mediated cross-contamination of LMF.

Method: The polypropylene (PP) surface was coated using a two-step coating method that employs zein as a polymeric binder for the chlorinated yeast cells. The antimicrobial activity of the coated PP surfaces was evaluated against *Escherichia coli* O157:H7 and *Listeria innocua* using a plate count assay. The EPA's sponge-based abrasion test was performed to assess the mechanical resistance of the antimicrobial coating in the simulated processing environment. The efficacy of the coated PP surfaces in reducing the surface-mediated cross-contamination of LMF was tested against *Enterococcus faecium* using wheat flour as a model LMF.

Results: The coated PP surfaces showed strong antimicrobial activities against *E. coli* O157:H7 and *L. innocua*, and the bacterial populations inoculated on the coated surfaces (ca. 6.53 – 6.68 log CFU/cm^2) decreased below the detection limit (1.0 log CFU/cm^2) within 10 min. Based on the sponge abrasion test, the antimicrobial coating formed on PP surfaces showed strong resistance against mechanical abrasion without losing its antimicrobial activities. The coated PP surfaces significantly ($p < 0.05$) reduced the surface-mediated cross-contamination of wheat flour. The cross-contamination levels on a coated PP surface were reduced by approximately 2.3 logs, compared to the control sample (ca. 6.1 log CFU/g), i.e., fresh wheat flour in contact with an uncoated surface contaminated by the inoculated wheat flour (ca. 7.84 log CFU/g).

Significance: These results demonstrate the potential of a food-grade biopolymer-based antimicrobial coating on LMF-handling surfaces to reduce the risk of microbial cross-contamination of LMF.

P3-91 Application of Non-Thermal High Voltage Atmospheric Cold Plasma (HVACP) Technology to Increase Shelf Life of Fresh Strawberries

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✦ ***Undergraduate Student Award Entrant***

Introduction: Fresh strawberries are susceptible to *Botrytis cinerea* mold spoilage leading to significant economic losses in production and only a seven-day post-harvest shelf-life. To extend the shelf-life of fresh strawberries, this study employs the novel non-thermal High Voltage Atmospheric Cold Plasma (HVACP) technology to remove dormant *B. Cinerea* spores from the surface.

Purpose: Evaluate the ability of a cold plasma treatment to significantly reduce *B. cinerea*, leading to an increased shelf-life of fresh strawberries, while retaining their desired consumer qualities.

Methods: 25g of fresh strawberries were spot inoculated with 6 $\log_{10}$ CFU/g of *Botrytis cinerea* mold spores and stored at 4℃ overnight. Subsequently, they underwent indirect HVACP treatment with optimized conditions (80 kV, 5 minutes, air, 100% RH, 175W, and 24h post-storage treatment). Microbial counts and quality assessments (color, firmness and pH) were performed on refrigerated samples on days 0, 2, 5, 7, 10, and 12. Measurements were conducted in triplicates and data analyzed using one-way ANOVA and Tukey's post-hoc test ($p \leq 0.05$).

Results: Treated samples exhibited a significant reduction (99.6%) in mold population compared to untreated samples. No differences in color, firmness, and pH was observed between treated and untreated samples over the 12-day study period. For untreated samples, molds were observed by day 7, whereas for treated samples, mold growth was first observed on day 12.

Significance: The study demonstrates that HVACP treatment significantly enhances the shelf-life of strawberries without compromising quality. This treatment effectively reduces *B. cinerea* spores, controlling mold growth and decay over 12 days without inducing thermal inactivation. These findings suggest potential applications of HVACP across various fresh produce, revolutionizing post-harvest handling and storage practices in agriculture.

P3-92 Mitigating Food Waste: A Sustainable Solution through Biodegradable Silk Coating with Antimicrobial Properties for Improved Food Safety and Extended Shelf Life

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Introduction: In response to the pressing challenges posed by global population growth and escalating food waste, this study introduces a novel approach to enhance food safety and extend the shelf life of perishable items.

Purpose: The primary objective is to address food safety concerns and combat food waste by developing an innovative and sustainable biodegradable silk coating embedded with antimicrobial properties.

Methods: We extract silk fibroin, a natural protein polymer, from silkworm cocoon waste—a byproduct typically overlooked by the textile industry. This forms the basis for our environmentally friendly antimicrobial food packaging solution. Utilizing ultrasonication, we encapsulate antimicrobial essential oils within silk nanoparticles, with no need for solvents or surfactants. The solution underwent testing for shelf life at room temperature and evaluation of its antimicrobial effects on *Listeria monocytogenes* and *Salmonella* Typhimurium.

Results: The developed edible silk coatings not only serve as effective barriers against gases and moisture, preserving the freshness of perishable foods like cherry tomatoes, but also contribute to an improved shelf life for coated samples at room temperature. Their transparency, easy washability, and residue-free nature make them a practical choice. With proven antimicrobial properties, these coatings efficiently inhibit the growth of foodborne pathogens, providing extended protection. Additionally, the dried film delivery method enhances stability, making transportation easier and offering a promising solution for developing countries with limited access to refrigeration or electricity.

Significance: This research contributes to the development of functional agri-food materials through scalable manufacturing, focusing on harnessing biodegradable polymers. The biodegradable antimicrobial silk coating emerges as a sustainable alternative to traditional plastic packaging, offering extended shelf life, improved food safety, and reduced waste. The innovative solution presented in this study represents a scalable and sustainable approach to addressing food insecurity, minimizing food waste, and mitigating climate change, thereby promoting a more sustainable planet.

P3-93 Viability of Modified Atmosphere Packaging (MAP) and Ultra-Violet C Radiation (UV-C) Exposed *Listeria innocua* NRRL B-33016 and *Escherichia coli* ATCC 25922 on Strawberries *(Fragaria x ananassa)* During Refrigeration Storage

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❖ ***Developing Scientist Entrant***

Introduction: Modified atmosphere packaging (MAP) and ultraviolet C radiation (UV-C) were investigated as ways to reduce the viability of pathogenic bacteria on strawberry surfaces.
Purpose: Determine the efficacy of modified atmosphere packaging and ultra-violet C dosage on the viability of *Listeria and E. coli* on the strawberries' surfaces and on other microorganisms, and the effect on the quality parameters of strawberries during refrigerated storage after treatment.
Methods: Fresh strawberries (4 to 6 per batch) were spot inoculated with *Listeria innocua* B-33016 (4.51 logs CFU/g) and *Escherichia coli* ATCC 25922 (4.18 logs CFU/g) on a sterile petri dish and placed in polypropylene tray. The trays were sealed with MAP (10% O_2, 15% CO_2, and 75% N) and refrigerated at 4°C for 12 days. Strawberries were analyzed for both microbial and physicochemical parameters at three-day intervals. Six treatments were analyzed: untreated strawberries (Ctrl), strawberries treated with 15 Joules cm^{-2} UV-C, atmosphere packaged strawberries with air as working gas (FA), FA combined with 15 Jcm^{-2} UV-C (FUA), MAP packed strawberries (MAP), MAP combined with 15 Jcm^{-2} UV-C (UMAP). All treatments were performed in triplicates; mean separation was analyzed using ANOVA and Tukey's studentized range test at α=0.05.
Results: *Escherichia coli* counts ranged from 2.60±0.39 log CFU/g (FUA) to 4.18±0.35 (Ctrl) immediately after treatment, while *Listeria innocua* counts ranged from 1.65 ± 0.16 log CFU/g (15 Jcm^{-2} UV-C) to the 3.63±0.19 (UMAP) at day 1. On day 12, the highest and lowest percentage weight loss recorded were 20.08±0.00% (Ctrl) and 0.88±0.02% (UMAP) respectively. O_2 content ranged from 16.70±0.00% to 0.10±0.00% (day 0 to day 12 respectively) while CO_2 content ranged from 1.67±0.05% to 55.63±0.05% (day 0 to day 12 respectively). No significant difference (p>0.05) was observed for color and texture during storage.
Significance: This study demonstrated that the modified atmosphere packaging with UV-C exposed strawberries could reduce the viability of *Listeria, E. coli*, aerobes, yeast, and molds on strawberries.

P3-94 Microbial Community Dynamics in Fresh and Treated Ready-to-Eat Vegetables during Storage

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Introduction: As consumption of ready-to-eat (RTE) vegetables continues to increase, there is a pressing need to develop novel strategies to simultaneously improve the safety and extend the shelf-life of RTE vegetables without altering their nutritional and sensory aspects.
Purpose: This study aimed to compare the microbial dynamics of fresh and treated vegetable products (e.g., juice) during storage.
Methods: Fresh spinach samples were blended with deionized water (1:1, w/w). Each sample was divided into three aliquots, which were either pasteurized (72ºC, 15s), treated with gallic acid (8 mg/mL), or untreated, and stored at 4ºC and 15ºC for 7 days. Samples were collected daily for 16S rRNA amplicon sequencing using culture-independent and culture-dependent approaches. DNA was extracted with the Qiagen DNeasy Kit and paired-end sequencing (2 x 300) was performed on the Illumina MiSeq. Raw reads were preprocessed and analyzed using bioinformatics pipelines.
Results: Culture-independent 16S rRNA amplicon sequencing yielded more bacterial genera (n=28) than the culture-dependent 16S rRNA amplicon sequencing approach (n=18). Higher abundance of *Pseudomonas*, *Exiguobacterium* and *Erwinia* was observed in untreated samples. *Exiguobacterium* abundance decreased with an increase in Enterobacteriaceae during storage. Microbial diversity increased during storage with genera including *Aeromonas, Chryseobacterium*, *Delftia*, *Enterococcus*, *Pectobacterium*, and *Shewanella,* as well as Enterobacteriaceae detected in untreated samples. Pasteurization inhibited *Erwinia*, *Exiguobacterium*, *Paenibacillus,* and *Pseudomonas,* but promoted the enrichment of *Bacillus* in the samples. Conversely, in gallic acid-treated samples, abundance of the bacteria genera (except *Pseudomonas*) was reduced throughout storage. The two treatments reduced and/or inhibited *Aeromonas*, *Chryseobacterium, Delftia,* Enterobacteriaceae, *Exiguobacterium, Paenibacillus,* and *Sphingobacterium,* which contain pathogenic and/or spoilage microbial species.
Significance: RTE vegetables are highly perishable and may serve as reservoir for foodborne pathogens. While pasteurization and gallic acid proved effective in inhibiting microbial communities associated with foodborne illness or spoilage during storage, a combination of these treatments would improve food safety and extend shelf-life to reduce food waste.

P3-95 Changes in Microbial Community Composition of Spinach from Harvest to Shelf Life

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❖ ***Developing Scientist Entrant***

Introduction: An improved understanding of changes in the microbial community composition of baby spinach over shelf life may help identify key genera that drive microbial spoilage of baby spinach, contributing to food loss and waste throughout the produce supply chain.
Purpose: The goal of this research is to characterize the bacterial and fungal community compositional changes on baby spinach over shelf life and by location to facilitate improved methods for shelf-life prediction and enhancement.
Methods: Samples were collected from lots of baby spinach grown in Yuma, AZ/Imperial Valley, CA (n=102) and Salinas, CA (n=138), over the course of one year. DNA was extracted from unwashed harvest sample and corresponding packaged baby spinach samples over shelf life at 4ºC at six different timepoints post commercial washing. 16S rRNA gene sequencing was performed using the Illumina MiSeq. DADA2 was used to taxonomically classify 16S rRNA gene amplicons. Data analysis was performed in R Studio with dplyr, phyloseq, ggplot2 and vegan packages and Kruskal-Wallis and PERMANOVA tests.
Results: The three most common bacterial genera identified across samples and time points were *Pseudomonas*, *Pantoea*, and *Exiguobacterium*. *Pseudomonas* relative abundance differed significantly with respect to timepoint and region (p<0.05) with the relative abundance of this genus increasing over shelf life; *Buchnera* relative abundance also differed by timepoint. PERMANOVA using the Bray-Curtis dissimilarity matrix, when constraining the permutation by month and lot, indicated timepoint was a significant variable (p<0.001), with an R^2 of 14.4% and 11.7% respectively, indicating overall changes in microbial diversity over time.
Significance: This research reveals potential microbial drivers of spoilage in baby spinach. Charting changes in the most prevalent genera over shelf life can inform future research and/or industry approaches to managing the microbial quality of baby spinach.

P3-96 Assessing and Modeling Bacterial Growth on Baby Spinach Sourced from a Supply Chain in China

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❖ ***Developing Scientist Entrant***

Introduction: China is the largest producer of spinach worldwide. Characterizing bacterial levels on spinach from a supply chain in China and using these data to evaluate a model that predicts bacterial growth on spinach, can inform management practices for this commodity.

***Purpose*:** This study aims to collect data on bacterial levels on spinach from a supply chain in China and use these data to:(i) identify factors that influence bacterial levels on spinach, and (ii) assess the accuracy of a previously developed Monte Carlo (MC) simulation for predicting bacterial growth on spinach from this supply chain.

Methods: Spinach, sampled over April–June 2021, was tested after harvest and packaging. Packaged spinach was stored at 4°C for shelf-life testing (until day 10, D10); shelf-life testing included packaged spinach that was distributed through retail or eCommerce channels. Aerobic plate count (APC) data was obtained by plating samples onto aerobic count Petrifilm, which was incubated at 35˚C for 48 h. A subset of these data (i.e., bacterial concentration on day 0 of shelf life, D0), and previously published microbiological data from this supply chain, were utilized to parametrize a MC simulation of bacterial growth on spinach.

Results: The median APC (interquartile range, IQR) of the spinach (n=11) on D0 was 6.73(6.52-7.18) $\log_{10}$CFU/g. On D10, the median (IQR) APC of spinach distributed via retail (n=11) or eCommerce (n=8) was 7.95(7.37-8.78) and 7.39(7.25-8.59) $\log_{10}$CFU/g, respectively; APC on D10 was not significantly different (p>0.05) by distribution channel. The median (IQR) concentration on D10, predicted by the MC simulation, was 7.97(7.76-8.32) log CFU/g.

Significance: For this supply chain, distribution channel alone did not explain bacterial levels on spinach, indicating the need to identify other factors that impact bacterial levels. The MC simulation's predictions appear to agree with the experimental data, indicating that it can be used to identify other factors that may impact bacterial levels (hence, shelf life) in this supply chain.

P3-97 Survival of *Escherichia coli* O157:H7 in Pico de Gallo and Lemon-Parsley Vinaigrette

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❖ ***Developing Scientist Entrant***

Introduction: This study investigates the survival of *Escherichia coli* O157:H7 in pico de gallo and lemon-parsley vinaigrette. Prompted by past outbreaks, including the 2008 *Salmonella* Saintpaul outbreak linked to produce and a 2023 Swedish outbreak tied to parsley, this research addresses critical concerns in food safety, examining the survival dynamics of *E. coli* O157:H7 in these foods that are consumed raw.

Purpose: This study investigates *E. coli* O157:H7 survival in pico de gallo (4°C and 10°C, for 15 days) and Lemon-Parsley vinaigrette (4°C and 25°C, for 7 days) at two pH levels.

Methods: Pico de gallo (25g), consisting of white onion, roma tomatoes, salt, cilantro, lime, and jalapenos, was inoculated with a pool of five GFP labelled *E. coli* O157:H7 isolates, incubated at 4°C and 10°C for 15 days. Lemon-parsley vinaigrette (25ml), consisting of olive oil, white wine vinegar, lemon zest, lemon juice, salt, grainy mustard, and parsley, was also inoculated with the *E. coli* O157:H7 pool. Incubation at 4°C and 25°C occurred over 7 days, considering pH variations.

Results: At 4°C and 10°C, one log CFU/g reduction was observed in pico de gallo after 15 days post inoculation. Pico de gallo's pH (3.5–4) remained stable, showing no significant differences with or without jalapenos. In lemon-parsley vinaigrette, complete bacterial inactivation occurred at 4°C and 25°C by day three. However, when the pH was raised to 3.7, bacterial growth could still be detected at day 7.

Significance: *E. coli* 0157:H7 is not inactivated by the ingredients of pico de gallo when stored at 4 and 10°C for 15 days. Vinaigrette at low pH can kill *E. coli* by day 3 after inoculation and can be detected for up to 7 days if the pH increases. Preparation of these salsas and vinaigrette should be done with proper hygienic conditions, as *E. coli* can survive for extended periods in these matrices and could cause illness to the consumer.

P3-98 Microbial Inactivation in Cold-Filled Acidified Foods

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Introduction: Acid-resistant foodborne pathogens have led to numerous outbreaks in acid foods having pH values below 4.0, thus raising concerns about the safety of these products. Commercial sterility of five-log reduction highly resistant Listeria species in shelf-stable acidified foods is commonly achieved through hot-filling, but cold-fill-hold processes possess promising potential if microbial safety and stability is ensured.

Purpose: Determine the time required for a five-log reduction in *Listeria innocua* in commercially prepared apple juice.

Methods: Store-bought commercially sterile apple juice was independently inoculated with *Listeria innocua* and kept at ambient temperature (25°C) to determine the holding times needed to achieve a five-log reduction in *Listeria innocua*. Trials were performed in triplicate followed by microbial count determination at sampling times. Samples were enumerated every 2 days up to 8 days.

Results: A cold-fill-hold process provided a 90% (one-log) reduction in *L. innocua* in the samples with a hold time of 3.29 days at 25°C (R^2=0.28). Greater than five-log reductions of pathogenic bacteria were estimated at 16.5 days after inoculation.

Significance: Future work will include evaluation of conditions to provide a five-log reduction on pathogenic Escherichia coli O157:H7, Salmonella enterica, and Listeria monocytogenes with a formal shelf-life analysis for cold-fill-hold process to be selected as a thermal processing alternative.

P3-99 Identification and Characterization of Spoilage Microorganisms Isolated from Pasteurized Apple Juice with Atypical Defects

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Introduction: We observed an unusual defect, presumably pectin gels, in spoiled apple juice that was pasteurized and treated with 238 ppm dimethyl dicarbonate (DMDC).

Purpose: We aimed to identify and characterize the microorganisms linked to this defect.

Methods: Routine enumeration was performed, and bacterial identification of the isolates was based on 16S rRNA gene sequencing. Isolates were grown in nutrient broth (50 g/L glucose and 20 g/L yeast extract) in a shaking incubator at 30 ºC and cultured over 72 hours to determine growth kinetics of the isolates. Growth models were estimated from experimental data using 'biogrowth' R package. Lag time was fixed at zero hours. Microtiter broth dilution method was performed to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of DMDC against *Acetobacter* species in apple juice.

Results: The spoiled product had a high microbial load (4.98 log CFU/mL) and was identified as *Acetobacter* as the predominant spoilage microorganism in the sample. Two isolates, identified as *A. lovaniensis* and *A. fabarum* by 16S rRNA gene sequencing, were further characterized for their growth characteristics and resistance to DMDC. The growth characteristic of *A. fabarum* was best described by the logistic model with following parameter estimates: C=2.28, μ=0.189, and $\log N_0$=6.23. The growth characteristic of *A. lovaniensis* was best described the Baranyi model with following parameter estimates: $\log N_{max}$=8.71, μ=0.194, $\log N_0$=6.91. The MIC of DMDC against both isolates was 250 ppm. However, this concentration could not reliably prevent spoilage.

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We found that when the microbial load is high (6 log CFU/mL), the MBC of DMDC against both isolates was 500 ppm, which is above the limit of DMDC concentration legally permitted in beverages.

Significance: Our findings emphasized the importance of sanitation of juice processing equipment to prevent post-pasteurization contamination of *Acetobacter* which exhibits an enhanced tolerance to DMDC.

P3-100 Microbial Safety Assessment of Cold Brew Coffee

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❖ ***Developing Scientist Entrant***

Introduction: The microbiological safety of cold brew coffee has not been studied closely, however there are reported cases of contamination in store retail settings.

Purpose: Determine if cold brew can sustain growth of select pathogens and infer the influence of different parameters such as temperature abuse and additives by monitoring microbial counts, pH, and titratable acidity (TA).

Methods: Cold brew coffee samples were brewed at a 1:8 ratio at 4°C with (n=9) or without additives (cinnamon, nutmeg, and shredded coconut) (n=9) prior to inoculation with select foodborne pathogens (*L. monocytogenes*, *S. enterica*, *E. coli*, and *B. cereus*) at a concentration of 6-log. The cold brew samples were stored at 4°C or subjected to temperature abuse conditions (25°C and 37°C). Uninoculated cold brew samples served as a negative control, while inoculated 1X PBS samples were used as a positive control. Microbiological sampling was conducted every third day, where 10 ml aliquots were collected until Day 15. Three biological and technical triplicates were performed for each experiment. Enrichment procedures were implemented when plating fell below detectable limits. TA and pH were taken at each sampling event.

Results: Under refrigeration, viable cells were not recovered after Day 12 for *E. coli*, *L. monocytogenes,* and *S. enterica*. Days 0-9 were significantly different for all three pathogens (p<0.001). For all three pathogens, no growth was observed and the addition of nutmeg increased deactivation rates. Coconut allowed the population of *E. coli* to persist past Day 9. *B. cereus* populations did not decrease below 4.89 log. Significant growth of *B. cereus* was observed in plain and coconut samples from Day 9-Day 12 and Day 6-Day 9, respectively (p<.001). Initial pH ranged from 5.4 to 5.6 and decreased over time, whereas TA increased over time (p<.05).

Significance: Information on the survivability of pathogens in cold brew will help contribute to the standardization of cold brew handling and food safety practices.

P3-101 Contamination of Shelf-Stable Buttermilk Containing Salad Dressing by Lactic Acid Bacteria Can be Controlled Using Fermentates from Cultured Non-Fat Dry Milk

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Introduction: Microbial spoilage of shelf-stable salad dressings by lactic acid bacteria still occur sporadically, despite best efforts of employing hurdle technology and chemical preservation.

Purpose: To determine whether fermentates from cultured non-fat dry milk can control lactic acid bacteria (LAB) found in shelf-stable salad dressing containing buttermilk.

Methods: Contaminating bacteria from bloated dressing samples were isolated and four unique LAB were identified by MALDI-TOF. A cocktail containing all four LAB was used for challenge studies with buttermilk containing salad dressing produced with either 1% cultured non-fat dry milk (MICROGARD© 430), 600 ppm nisin preparation, or with no additional antimicrobials at a final concentration of 3.38 x 10^2 CFU/gram. A shelf-life study was also performed with uninoculated samples. Each study was sampled at 19 different time points over 300 days.

Results: In the challenge study, control samples with no added antimicrobials demonstrated logarithmic growth, reaching the highest levels of recoverable LAB at day 42 (7.89 log CFU/g), at which time the bottles exhibited bloating. Samples containing 1% cultured NFDM did not demonstrated growth or bloating, as the highest number of recoverable LAB were 2.66 log CFU/g (day 49) and then fell below the limit of detection (<10 CFU/g) on day 90 and remained below LOD for the remainder of the study. The samples containing nisin preparation remained below LOD 5 days post inoculation. For shelf-life testing, samples were held at 27°C and only the negative control samples exhibited growth and peaked at 5.6 log CFU/g on day 128. The pH values of the control, cultured NFDM, and nisin preparation samples during the study were on average 3.60(±0.16), 3.67(±0.04), and 3.66(±0.04) and water activity averaged 0.90(±0.01), 0.89(±0.01), and 0.89(±0.01), respectively.

Significance: Fermentates from cultured non-fat dry milk can be used as an effective hurdle against LAB contamination in buttermilk containing salad dressings.

P3-102 Identification of Spoilage Fungi Relate to Cultured Dairy Products Using Amplicon Sequencing

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❖ ***Developing Scientist Entrant***

Introduction: Identification of specific fungi, rather than relying solely on total yeast and mold counts, would aid the cultured dairy industry in reducing spoilage. However, there are technical challenges in primer selection and sequence analysis.

Purpose: This study explores whether common fungi can be identified to the species level by sequencing single amplicons. Moreover, alternative primers were developed to improve accurate species identification.

Methods: Sanger sequencing was conducted with primers targeting the ITS region (ITS5/ITS4), β-tubulin (Bt2a/Bt2b), calmodulin (CMD5/CMD6), and the translation elongation factor 1 regions (EF1/EF2 and TEF1728F/TEFLLErev). PCR with each primer set was performed on 11 fungal isolates representing 10 genera. Customized β-tubulin primers were designed to increase sequencing success rates. Both ITS and β-tubulin primers were used in PCR reactions on 114 fungal isolates from cultured dairy products. BLAST searches were conducted, and phylogenetic trees were constructed from sanger sequencing results using Mr. Bayes algorithm.

Results: All 11 representative isolates were sequenced with ITS5/ITS4 while sequences from PCR reactions using Bt2a/Bt2b, CMD5/CMD6, EF1/EF2 and TEF1728F/TEFLLErev only resulted in 5, 2, 2, and 7 sequences, respectively. TEF1728F/TEFLLErev reactions yielded the longest amplicons (938 ± 89 bp), while Bt2a/Bt2b reactions resulted in the shortest (373 ± 48 bp). Of the 114 fungal isolates, more than 98% were sequenced using ITS primers while only around 50% were sequenced using β-tubulin primers. BLAST searches using ITS sequences resulted in multiple 100% matches with type strains, so phylogenies were used to identify species. The β-tubulin primers did not result in more sequence types than ITS.

Significance: While the ITS sequence is the best choice for initial fungal identification, sequencing additional loci can enhance the resolution of identification of fungi from the cultured dairy industry.

P3-103 Applying Machine Learning to Predict the Types of Fluid Milk Spoilage

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❖ ***Developing Scientist Entrant***

Introduction: Diagnosing the fluid milk spoilage type (i.e., sporeformers or Gram-negative bacteria) is critical for suggesting intervention strategies, therefore a more consistent and efficient approach is needed to replace time-consuming expert review process.
Purpose: This study aimed to develop a machine learning classification model that predicts the type of fluid milk spoilage based on microbiological data for different days of shelf life.
Methods: Standard plate count (SPC) and enumeration data on crystal violet tetrazolium agar (CVTA) were collected on multiple days of shelf life (i.e., day 7, 14 and 21 after pasteurization) for 769 fluid milk samples. These data were reviewed by three experts and classified into three distinct categories of fluid milk spoilage, including spoilage due to (i) post pasteurization contamination (PPC), (ii) sporeformer growth and (iii) no microbial spoilage. These data were split based on when samples were collected; 321 samples were used for training and validation, and 448 samples were used for testing model prediction. Different sets of data at different days of shelf life were separately used to develop random forest classification models to investigate the effect of minimizing the number of tests on the model performance of predicting the spoilage type.
Results: The final model, which included microbiological data from days 7, 14, and 21 of shelf life, was validated with 95% accuracy and showed 97% prediction accuracy with the test set. When microbiological data on day 21 were excluded for model development, the validation and test accuracy were reduced to 79% and 89%, respectively. The variable importance plot showed that two most important predictors of the comprehensive model were CVTA counts on day 21 and 14.
Significance: This model serves as a digital tool that can be utilized for quick spoilage diagnostic testing and test interpretations, reducing the need to rely on expert interpretation.

P3-104 A Comprehensive Digital Tool to Predict Fluid Milk Spoilage

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Introduction: Post-pasteurization contamination (PPC) with Gram-negative bacteria and growth of sporeformers are two major causes of microbial milk spoilage, typically resulting in detectable sensory defects when bacterial concentrations exceed 6 log10 CFU/mL.
Purpose: This study aimed to develop and validate a model simulating the growth of Gram-negative bacteria and sporeformers in contaminated high-temperature short-time (HTST) milk along a supply chain, predicting milk shelf-life.
Methods: The milk processing plants in Voluntary Shelf-Life (VSL) Program were classified as Good, Medium, and Bad based on PPC-related spoilage percentages. Growth parameters for 17 Gram-negative bacteria and 9 sporeformers were estimated using the Baranyi model with 6°C growth data. Time-temperature profiles for 100 hypothetical HTST milk lots were simulated. Shelf-life predictions were made by calculating the percentage of milk containers exceeding 6 log10 CFU/mL. The model was validated using VSL Program data, comparing simulated and observed bacterial counts. The effect of consumer home storage temperature on milk shelf-life was also assessed.
Results: The model predicted shelf-lives of 26, 13, and 8 days for HTST milk from Good, Medium, and Bad plants (i.e., when 26% of containers exceed 6 log10 CFU/mL). Validation showed the model accurately predicted PPC and sporeformer counts. For PPC bacteria, simulated and observed percentages of containers over 6 log10 CFU/mL were 35.43% vs. 17.37% on day 7, 88.96% vs. 87.39% on day 14, and 98.97% vs. 90.13% on day 21. For sporeformers, the percentages were 0.2% vs. 0% on day 7, 3.87% vs. 5.29% on day 14, and 13.14% vs. 29.32% on day 21. Simulating home storage temperatures <4°C extended the shelf-life of Good plant milk by 7 days but had no impact on Medium and Bad plant milk.
Significance: This model presents a comprehensive milk spoilage prediction tool that can facilitate shelf-life prediction and assessment of intervention strategies for shelf-life extension.

P3-105 Culturable Microflora in Swine Guts

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Introduction: Swine are valuable large animal models for not only human biomedical applications but also for dietary exclusion of pathogenic microorganisms from the human guts. For any research that needs swine as an animal model, understanding the baseline levels of beneficial and pathogenic microorganisms in the swine guts is imperative.
Purpose: This study defined the baseline levels of various culturable microbial species in the swine guts.
Methods: Domesticated barrows (castrated male swine; *sus scrofa domesticus*) were used in three independent trials of the study. In each trial, the swine (n = 3) with an average individual weight of 118 kg were housed in a secured, climate-controlled environment that had individual pens (~3.9×1.5 m), each with solid (1.5 m) and slated floors (2.4 m), a feed bin, and automatic waterer. The swine were randomly allocated to individual pens and fed a commercial grower meal containing 14% crude protein, 5% crude fat, and 4% crude fiber. Fecal samples (approx. 50 g) from each swine were collected once a month and transported to our laboratory under freezing conditions. The populations of beneficial and pathogenic microorganisms in 1 g of each well-mixed fecal sample were analyzed using growth media selective for each group of microorganisms.
Results: Results showed that the populations of two beneficial bacteria, *Lactobacillus* and *Bifidobacterium* were 7.4±0.5 and 3.2±0.9 log CFU/g, respectively. Pathogenic bacterial populations, including those of *Bacteroides*, *Clostridium*, *Enterobacteriaceae*, *Staphylococci*, and *Streptococci* were 3.5±0.7, 7.9±0.1, 6.3±1.3, 5.9±0.4, or 8.8±0.5 log CFU/g, respectively. The total anaerobic population was 9.2±0.5 log CFU/g, whereas the populations of non-sporing anaerobes and gram-negative anaerobes were 8.7±0.5 and 8.0±0.5 log CFU/g, respectively. The total facultative anaerobes were 6.5±1.0 log CFU/g.
Significance: The study provides important baseline information for research that uses swine as an animal model to study the dietary influence on beneficial and pathogenic bacterial populations in human guts.

P3-106 Characterization of Microbiota in Plant-Based Meat with Extended Shelf Life

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Introduction: Plant-based meat is minimally processed, nutrient-rich, and susceptible to microbial spoilage, necessitating antimicrobials to extend the shelf life. However, the microbial dynamics of spoilage are not well characterized in plant-based meat because of their diversity and the deficiency of specific selective media for isolation. Microbiota characterization enables the processors to identify spoilage issues and apply targeted interventions.
Purpose: To determine the efficacy of a natural, clean-label antimicrobial, DuraShield™ (combination of cultured dextrose and rosemary extract), on the shelf-life extension of plant-based patties. Additionally, by performing next-generation sequencing, investigate diverse growth patterns of microbial ecology in plant-based patties.
Methods: Plant-based patties were prepared using pea proteins. Two treatments were included in the study- control (without antimicrobials) and test (with DuraShield™ at 1%)- and stored at 4°C for 21 days under aerobic packaging. Samples were assessed weekly for aerobic bacteria and lactic acid bacteria. The experiments were repeated twice with duplicate samples (n=4). Additionally, 16S rRNA gene profiling was performed from sample homogenates to characterize spoilage microbiota (n=6).
Results: Spoilage microbes grew extensively in the control patties, with the aerobic bacteria and lactic acid bacteria counts reaching 7 log within 7-10 days, leading to spoilage of the patties. However, DuraShield™ suppressed the growth of aerobic bacteria and lactic acid bacteria in plant-based patties

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until day 21, with microbial counts remaining below 6 log CFU/g (p<0.05). Moreover, 16S rRNA gene profiling of plant-based patties revealed the differential distribution of specific microbiota between control and test groups. Specifically, DuraShield™ inhibited the growth of key spoilage formers such as Pseudomonas, Carnobacterium, and Serratia in plant-based patties(p<0.05).

Significance: DuraShieldTM successfully controls spoilage in plant-based patties and extends the shelf life to an additional 10 days compared to the control.

P3-107 Waste Not, Want Not: An Exploratory Study of Food Donation Systems and Safety in Central Pennsylvania

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Introduction: Food waste and food insecurity are major problems in the United States; it is estimated that 40% of our food supply is wasted and 42 million Americans face food insecurity. Numerous issues contribute to these problems including a lack of unified guidelines for donation of foods and little research into the microbial quality of donated foods.

Purpose: This project addresses knowledge gaps and microbial safety issues in food donation by conducting 1) a needs assessment throughout the food donation stakeholders and 2) a microbial assessment of donated foods.

Methods: Aim 1) A series of personal interviews were conducted in Centre County, Pennsylvania for: food retailers, food banks/distributors, and consumers that obtain donated foods. Qualitative thematic analysis was used to assess what products and procedures posed the highest risks for the safety and quality of donated food items. Aim 2) Traditional plate-based culturing (Enterobacteriaceae, yeast, and mold) and next generation sequencing of food microbiomes (amplicon sequencing of the V4 region of the 16S gene via the Illumina platform) were used to determine how colony counts, and relative abundance of microbes are related to the age and storage conditions of food products.

Results: Preliminary results demonstrated that retail stores often lack written guidelines for food donation safety and quality parameters. Small food banks/distributors also lacked written guidelines and sufficient cold storage capacity for time/temperature control for safety (TCS) foods. Consumers were often confused about the meaning and safety implications of stated shelf-life dates. Microbial profiles may provide processors, retailers, distributors, and consumers with information to better address handling, storage, and shelf-life issues of donated foods.

Significance: These results highlight the food safety and quality issues present in the food donation system. Further research will inform the creation of learning materials to help address these issues for each group of stakeholders.

P3-108 Preservation of Cherries and Rice Seeds by *Eucalyptus camaldulensis* Essential Oil and Sweet Potato Starch-Based Edible Coating

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Introduction: Traditional food preservation techniques involve fungicides which are associated with various health hazards. There is dire need to formulate natural ingredient-based food preservation systems to extend the shelf life of fresh produce without compromising the health.

Purpose: The aim of this study was to develop eucalyptus essential oil (EO) based preservation system to control the fungal contamination in rice seeds and cherries.

Methods: Steam distillation was used for the extraction of EO from eucalyptus leaves and chemically characterized by gas chromatography and mass spectrometer. EO was evaluated for antibacterial, antifungal and antioxidant potential. Starch was extracted under optimized conditions from low-grade sweet potatoes by ultrasonic assisted extraction. Starch was modified and combined with EO to formulate active edible coating to control the fungal contamination in cherries and rice seeds. Moreover, EO was also used to control fungal contamination in rice seed inoculated with fungal spores.

Results: GCMS analysis of EO revealed the presence of 20 compounds with eucalyptol (60.159%) as predominant component. Free radical scavenging of 92% was observed by the highest test concentration of EO (250 mg/ml) and EO showed remarkable inhibition of both Gram positive (*S. aureus,* 36 ± 1 mm and *B. cereus,* 34 ± 1 mm) and Gram negative (*E. coli,* 30 ± 3 mm *and S. typhimurium,* 20.66 ± 3.05 mm) bacteria. *E. camadulensis* EO showed complete radial growth inhibition of *Aspergillus niger* and *Penicillium griseofulvum* at 25 µl/ml, which was marked as minimum inhibitory concentration. After a storage of 5 days, cherries coated with EO (3%) and modified starch showed significantly low (p<0.05) fungal contamination (40%) in comparison to control (95.5%). Whereas rice seeds treated with EO showed 0% contamination in comparison to control treatment.

Significance: Eucalyptus EO is a natural preservative and can be used to preserve the fresh food produce.

P3-109 Controlled Release Characterization of Hemp Loaded Electrospun Nanofiber and Antibacterial Activity for the Safety Enhancement of Raw Chicken Breast Meat

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❖ ***Developing Scientist Entrant***

Introduction: Hemp (*Cannabis sativa* sp) is a medicinal plant with bioactive phytochemicals, including phenolic acids, flavonoids, terpenes, and cannabinoids. The applications of bioactive components in food safety are limited due to their negative sensory impact. This could be circumvented by nanoencapsulating the active compound in electrospun nanofibers. The high surface-to-volume ratio characteristics of the nanofiber enhances the controlled release of the active compound and has potential for food safety applications.

Purpose: The objectives of the study were to characterize the controlled release profile while assessing its application in the safety of a packaged raw chicken breast model.

Methods: A novel active packaging nanofibrous film loaded with hemp extract into polyvinyl alcohol (PVA) polymer. Hemp loaded nanofibers (HNFs) were fabricated using the electrospinning technique with hemp extract loading concentrations of 0,7,9 and 13% (v/v). The controlled release profile, safety of the nanofiber film, and the subsequent assessment of raw chicken breast meat packaged in the film were investigated. Raw chicken breast fillets were cut into 10g cuboids, submerged into broth culture medium containing 10^5 CFU/mL (*Salmonella enterica* spp-SE and *Listeria monocytogenes*-LM), and allowed 20 minutes for cells to adhere. Prepared samples were dried, wrapped with electrospun nanofiber mats, and stored in petri dishes at 4°C for 96 hours. All treatments were analyzed in triplicate, and all statistical significance was tested at 5%.

Results: HNFs at 4°C showed 2.03 ± 0.17 log CFU/g and 0.89 ± 0.14 log CFU/g log reductions against LM and SE, respectively, indicating microbial growth inhibition effectiveness of the HNFs. Hemp-loaded nanofibers had an encapsulation efficiency range of 91-94%, a loading capacity of 92-99%, and a cumulative release of 14 -43% of encapsulated hemp extract into solution medium after 96 h.

Significance: Results suggest that HNFs have potential to augment the safety of raw poultry meat.

P3-110 Determinants of Antibiotic Residues Occurrence in the Rwandan Milk Value Chain

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Introduction: Milk and dairy products are recognized as an important source of animal proteins in the Rwandan diet. However, their quality and safety are generally compromised by the contamination of raw milk by antibiotic residues.

Purpose: A study was conducted to assess the factors associated with the occurrence of antibiotic residues in milk along the Rwandan dairy value chain.

Methods: A survey was conducted to identify the factors of milk contamination by antibiotic residues at the farm (n=144), transportation (n=150), milk collection centers (MCC) (n=54) and retail (n=48) levels by using structured questionnaire. Antibiotic residue detection was executed using Charm EZ kits targeting common dairy farming antibiotics (Tetracycline, Streptomycin, Beta lactams, and Chloramphenicol). The factors associated with the occurrence of antibiotic residues in raw milk were assessed by using a binary logistic regression analysis.

Results: The prevalence of antibiotic residues was found to be 29.9%, 48.0%, 74.0% and 41.7% respectively at the farm, transportation, MCC and retail levels. Tetracycline residues were most prevalent (82.0%), followed by Streptomycin (12.8%) and Betalactams (5.2%), while no Chloramphenicol residues were found in the analyzed samples. The occurrence of antibiotic residues in raw milk was found to be significantly ($p \leq 0.05$) associated with the farmer's noncompliance to the withdrawal periods following the antibiotic administration to dairy cows and the absence of adequate equipment and test kits for detecting the possible occurrence of antibiotic residues in raw milk during transportation and at MCC level.

Significance: This study highlights the potential risk to consumers posed by antibiotic residues in Rwandan dairy products. There is therefore a need to enhance the dairy farmer's knowledge on antibiotic use and farm hygiene. The testing capacity of milk transporters and MCC personnel to be able to detect antibiotic residues must be enhanced to ensure milk is safe for consumption.

P3-111 Developing Environmentally Friendly Food Containers and Packaging Materials with Waterproof Properties Using Rice Husk-Extracted Cellulose Combined with Gelatin

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Introduction: Disposable paper utensils, commonly used for beverages or takeout, pose recycling challenges due to the difficulty in separating the inner polyethylene film from the paper. The high cost associated with this process renders it economically unviable for most recycling facilities. Additionally, they often contain toxic chemicals such as polyfluoroalkyl substances (PFAS), raising environmental and human health concerns.

Purpose: We aim to incorporate edible materials and modify the gelatin formula to create a substance that can replace the plastic coating on paper cups or function as a waterproof freshness spray that contributes to food safety goals.

Methods: The film-forming formulation is created by cellulose (extracted from rice husk) with gelatin under different concentrations, acidic and alkaline conditions. Waterproof indicators, including contact angle and water droplet permeation time, are calculated. The optimal formulation was measured by waterproof tests such as immersion time, adhesive application frequency, and additional glycerol or alcohol. Furthermore, film degradation, heat resistance, puncture strength, and water permeability are tested. Subsequently, paper box containers are manufactured, and an evaluation is conducted by spraying apples to assess the extension of freshness.

Results: The optimal formulation in this study is as follows: 8% gelatin aqueous solution, glycerol, alcohol, and 40% rice husk extracted cellulose. The heat resistance of the optimal formulation paper film can reach up to 150°C. The optimal formulation paper film is used to create various paper containers with water permeability rate of 0.0% after one day. When applied to fruits, the formulation significantly extends the freshness period. Moreover, the cost of producing waterproof paper straws and paper boxes is low.

Significance: This product is an edible spray coating with the advantages of low consumption, convenience, and low cost. It can replace plastic coatings on the market, contributing to food safety and a cleaner environment for the Earth.

P3-112 Development of Active Food Packaging Films Using Bacterial Cellulose and Pullulan with Silver Nanoparticles

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial cellulose (BC) and Pullulan, derived from natural sources, are known for their mechanical strength and film-forming abilities. Combined with antimicrobials, they could inhibit microbial growth, ensuring food safety and quality in food packaging.

Purpose: This study investigated the combination of BC and pullulan with silver nanoparticles for the development of active food packaging films.

Methods: BC was produced by *Komagataeibacter xylinus* (ATCC 53524) in Hestrin-Schramm medium at 30°C for 21 days under static conditions and purified to obtain BC slurry. The film-forming solution (FFS) was developed by blending BC slurry (75% w/v and 85% w/v) with gelatinized pullulan (5% w/v) -glycerol (10% w/v) using ultrasonication at 600W for 1 minute at 65°C and dried for 24 hours at 40°C to obtain a nanocomposite film. The chemical and thermal properties of BC and BC-pullulan films were evaluated using different analytical techniques. Sterile discs of BC-Pullulan films were immersed in 1µM silver nanoparticles (AgNPs) for 1 hour and the zone of inhibition was examined against *E. coli* O157:H7, *Listeria monocytogenes, Salmonella, Staphylococcus aureus*. All the experiments were done in triplicate.

Results: BC-Pullulan discs didn't exhibit any inhibition against the pathogens. However, upon impregnating with AgNPs, the 75% BC-pullulan-AgNP formulation demonstrated significant inhibition ($p < 0.05$) against the growth of *E. coli* O157:H7, *Listeria monocytogenes*, and *S. aureus* by 22.16±0.10 mm, 19.67±0.15 mm, and 24.33±0.21 mm, respectively. Increasing the BC concentration to 85% in BC-Pullulan-AgNPs, resulted in an additional inhibition of growth of *Listeria monocytogenes*, and *S. aureus* by 2-3 mm. Both 75% and 85% BC-Pullulan-AgNPs showed no significant effect on the growth of *Salmonella*. FTIR analysis revealed distinctive O-H stretching vibrations in 75% and 85% BC-Pullulan samples, characterized by a broader peak specific to pullulan at 3276 cm^{-1}, contrasting the BC sample.

Significance: BC-pullulan films containing silver nanoparticles exhibit promising potential in the food industry as an active food packaging material.

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P3-113 Mitigating *Listeria monocytogenes* in Ready-to-Eat Deli Meat with an Edible Packaging Film Engineered Using Gelatin and Lactate Diacetate Compound

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❖ *Developing Scientist Entrant*

Introduction: Edible packaging film with chicken skin collagen to improve mechanical properties and addition of antimicrobial compounds can provide a substitute for plastic packaging.

Objective: Evaluate the mechanical and antimicrobial properties of edible packaging film engineered using chicken skin collagen and anti-listeria lactate diacetate compound against *Listeria monocytogenes* inoculated on deli meat over 12-weeks of refrigerated storage.

Methods: An edible film with base formulation of 3% Chicken Gelatin (CG) + 4% Nanocellulose (NC), starch (1%), glycerol (6%), and water (85-91%) was developed with either 0, 1 or 3% lactate diacetate (LD) treatments. The hand-cast films dried for 5 days were incorporated with LD and dried (5 days). Edible films were analyzed for tensile force, Young´s modulus, elongation, and punching force. For the antimicrobial analysis, films with three antimicrobial treatments (0, 1 and 3% LD) and one control treatment (without any additional packaging) were tested. Fully cooked, deli meat (beef bologna) was inoculated with ~6 logs of *L. monocytogenes*, the antimicrobial side of the film was laid on top of the inoculated deli surface, vacuum packaged, stored (4°C) and sampled weekly for twelve weeks (3 samples x 2 trials, n=240). Deli+film was aseptically placed in a sterile whirl pack bag, stomached (Buffer Peptone Water 1:1w/w, for 1min), serially diluted and spread plated (0.1 mL) on modified Oxford agar and incubated at 37°C for 24 hours. Viable colonies were counted and reported as log CFU/g. Data was analyzed using ANOVA with significant mean differences at $p<0.05$ (Tukey's HSD).

Results: Addition of LD to edible films with collagen significantly ($p<0.05$) reduced its mechanical properties. The LD packaging films initiated anti-listerial activities from week 4 with *L. monocytogenes* reducing by 2-3 logs at the end of 12-weeks.

Significance: Edible films with chicken skin collagen and LD offer a promising alternative for food packaging.

P3-114 Improving the Quality of Catfish Fillets by Using a Combination of Antimicrobial Treatment and a Gelatin Coating, Packaged in a Moisture-Control Tray

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❖ *Developing Scientist Entrant*

Introduction: Chlorine Dioxide (ClO_2) antimicrobial properties have been studied for many years. The application of ClO_2 in seafood products, gelatin coating, and using the technology of a moisture-control package, bring a potential solution to overcome the main challenges related to the limited shelf life of these products by controlling moisture, avoiding lipid oxidation, and inhibiting microbial growth.

Purpose: The purpose of this study was to evaluate the effect of gelatin treatment combined with an antimicrobial (ClO_2) packed in a moisture-control tray to extend the shelf life of thawed catfish fillets.

Methods: Fresh catfish fillets were separated into four groups (24 samples per treatment): Non-treated fillets as control (C), gelatin-coated (GX), antimicrobial with gelatin (AGX), and antimicrobial with gelatin in moisture control (AGM). All samples were frozen and stored in the freezer at ≤-20°C for 48 hours and then thawed overnight at ≤4°C for analysis. Physical/chemical and microbial activity were analyzed right after thawing and every four days for 20 days. Data obtained from different analyses and between treatments was analyzed with an analysis of variance (ANOVA) with Tukey's studentized range test ($\alpha = 0.05$).

Results: *Pseudomonas* spp. and psychrophilic bacteria counts were 3.08 ±0.40 and 3.95±0.29 Log CFU/g respectively at day 0 and showed no significant difference between groups. All spoilage microorganism counts remained under the recommended threshold until day 8. For TBARS analysis, GX, AGX, and AGM (0.34±0.12, 0.33±0.10, 0.36±0.16 mg MDA/Kg) displayed significantly lower values compared to CX (1.20±0.33 mg MDA/Kg) throughout the study. All treatments for TBARS values remained under recommended limits.

Conclusion: The combination of gelatin and ClO_2 does not enhance the shelf life of thawed catfish fillets regardless of the use of a simple or a moisture control tray. Gelatin coating prevents fat oxidation regardless of the packaging.

P3-115 Development of Visible-Light Responsive Antimicrobial Packaging Film with Dye-Sensitized TiO2 Conjugates to Enhance Food Safety

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❖ *Developing Scientist Entrant*

Introduction: Antimicrobial packaging materials hold promise in reducing bacterial growth in foods, but current antimicrobial packaging solutions face various challenges, such as the rapid exhaustion of antimicrobial agents and concerns of antibiotic abuse.

Purpose: Develop and evaluate a novel photocatalytic antimicrobial packaging material that can inhibit bacterial growth by generating reactive oxygen species (ROS) under visible light.

Methods: Photocatalysts TiO2-tetra(4-carboxyphenyl)porphyrin (TcPP) were synthesized by mixing TiO2 and TcPP in ethanol and stirred at 75 °C overnight. The conjugates were resuspended in water after ethanol removal, mixed with cellulose nanofibrils, and cast into films The film's log CFU reduction of *E. coli, Leuconostoc lactis, Pseudomonas fluorescens, Listeria innocua* (n=2) under different light intensities and durations was determined at 4°C. Log CFU/cm2 reductions of *E. coli* were determined on cucumbers in contact with the film exposed to 6000lux at 4°C.

Results: Overall, the effectiveness of the conjugate was impacted by bacteria type and light intensity ($p<0.05$). Under 3000 lux light, the film caused a 4.4-log reduction in *E. coli*, a 3.8-log reduction in *Leu lactis,* a 4.7-log reduction in *L. innocua,* and a 4.6-log reduction in *P. fluorescens* occurred after 72 hours. The antimicrobial efficacy decreased as the light intensity decreased. At a low light intensity of 600lux, the film resulted in a 4.2-log reduction in *L. innocua* and 1.0-log reduction in *L. lactis* but no significant reduction in *E. coli* or *P. fluorescens* ($p<0.05$) after 96 hours. Moreover, the film led to a 3.5 log CFU/cm2 reduction in *E. coli* on cucumber after 72-hour of 6000-lux light illumination.

Significance: This study designed a unique photocatalytic packaging film that can harvest low-intensity visible light energy to generate reactive oxygen species that inactivate microorganisms. The film has a great potential to reduce bacterial contamination in foods to improve safety and extend shelf life.

P3-116 Navigating the Microplastics and Nanoplastics Challenge with a Product Stewardship Framework

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Introduction: The scientific community has seen an increase in research focused on detecting, examining, and describing microplastics/nanoplastics (MNPs) in various food and drink products. These particles are typically a blend of shapes, chemical compositions, and adsorbed substances. This diversity, combined with gaps in our understanding of the potential hazards and dose-response relationships of MNPs, complicates application of traditional risk assessment. The public's perception is often influenced by the presence of MNPs, regardless of whether exposure is sufficient to cause adverse health

effects. As a result, companies that produce foods and beverages are expediting efforts to identify and eliminate the sources of MNPs from their products.

Purpose: Our objective is to provide a pragmatic, priority-based, vulnerability assessment framework for the food and beverage industry to identify potential sources of MNPs in the supply network.

Methods: Our approach is grounded in existing research related to food components and categories associated with MNPs. We conducted a review of peer-reviewed scientific literature using food science keywords and semantic search. Extracted data were grouped based on commonalities.

Results: A scoping review of the literature from the past 3 years yielded 6,100 primary and secondary publications. Employing ASReview software and human reviewers, we identified the top 15 food and beverage categories, such as seafood, meats, processed foods, produce, honey, sugar, salt, dairy, and bottled water. Supply network stratifiers of interest included food sourcing, processing levels, and packaging. This data and analysis ultimately produced adaptable decision-making pathways for various food and beverage contexts.

Significance: The detection of MNP particles alone does not imply any probability of adverse health effects. Their presence is nonetheless undesirable. This research provides manufacturers with a fundamental product stewardship framework to protect consumer health and reduce liability.

P3-117 Rain Splash-Mediated Dispersal of *Escherichia coli* from Fecal Deposits to Field-Grown Lettuce is Affected by Mulches

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Introduction: Mulch is used to maximize crop yield by suppressing weeds and modulating soil temperature and moisture levels. However, various mulches may differentially impact rain-mediated dispersal of bacteria from a fecal point source.

Purpose: Assess *E. coli* dissemination from a fecal point source to a lettuce crop grown on different mulches.

Methods: Loose-leaf lettuce 'Magenta' seedlings were transplanted (N=22/bed) into 28- randomized, 3 m long, raised beds with black plastic, biodegradable plastic, straw or left bare. Eleven days post-transplant, 10 g of rabbit manure spiked with ~8 log CFU/g *E. coli* TVS353 were deposited in each bed at the 0 m mark. One (N=12/bed) and three (N=10/bed) days after rain, lettuce was collected along 1.5 m transects on either side of fecal deposits and lettuce-associated *E. coli* semi-quantified with an MPN assay. Data were fitted to a Weibull Model to predict distances at which *E. coli* would diminish 3-7 log from level in feces.

Results: Both distance (p<0.001) and mulch (p<0.001) were factors for *E. coli* transfer from point source to lettuce. *E. coli* recovery differed by mulch type, with biodegradable plastic and straw yielding the largest difference (2 log, p<0.001). Mulch and distance were also significant factors in *E. coli* recovery 3-days post-rain (both p<0.001). In this trial, both plastic mulches differed from bare ground and straw (p<0.01). For every mulch, less *E. coli* was retrieved from lettuce at 0.3 m, 3 days post-rain compared to 1-day (p<0.01; all four mulches). Weibull modeling predicated a 7-log reduction in *E. coli* from fecal levels would be achieved at 1.2-1.4 m from point source on plastic mulch, 0.75 m on bare ground (p<0.05) and 0.43 m on straw (p<0.01).

Significance: Straw mulch and bare ground limited rain-mediated *E. coli* dispersal to lettuce compared to plastic mulches 1- and 3-days post-rain. These findings can inform recommendations for harvesting measures related to animal intrusion in vegetable production areas.

P3-118 *Salmonella enterica* Growth in Tomato Fruit Surface Washes is Affected by Types and Amounts of Different Sugars

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* can utilize plant surface metabolites to persist on crop surfaces. How sugar levels on tomato fruit surfaces change during ripening and how they modulate *Salmonella* dynamics remain unexplored.

Purpose: To investigate shifts in fruit surface sugars during tomato ripening and their modulatory effects on *Salmonella enterica* association.

Methods: Mature green and red ripe fruit of tomato cultivars 'Heinz-1706' and 'Black Icicle' were washed in sterile water for 90 min to collect surface metabolites and aliquots lyophilized and resuspended in 1.5 mL High Performance Liquid Chromatography (HPLC)-grade water. Samples were analyzed in duplicate for sugars using targeted HPLC-Mass Spectrometry (MS). Relative abundance (RA) of each sugar was calculated by integrating peak height. Data were analyzed by multiple regression and correlation analyses was conducted using *Salmonella* growth parameters (lag phase, growth rate, final population value) from growth curve analyses.

Results: Ripeness (p<0.01) was a factor for amount of glucose, fructose and sucrose in tomato fruit surface washes. Cultivar was a weak factor for fructose (p=0.05) and sucrose (p<0.1). Mature green 'Heinz-1706' wash had higher sucrose (p<0.05) and lower glucose and fructose than ripe 'Heinz-1706' (both p<0.01) and green/ripe 'Black Icicle' wash (all p<0.05). Sucrose was negatively associated with glucose (R=-0.9; p<0.05) and fructose (R=-0.9; p<0.01) in both cultivars. An 'unidentified' sugar peak, present in green fruit only, was higher (p<0.01) in 'Heinz-1706' than 'Black Icicle'. In 'Black Icicle', sucrose was strongly negatively correlated with lag phase (R=-0.81; p<0.05), and weakly with growth rate (R=-0.68) and final population (R=-0.55). Glucose was positively correlated with lag phase (R=0.95, p<0.01). In 'Heinz-1706', final population was correlated with glucose levels (R=0.76; p<0.1), and inversely associated with sucrose (R=-0.68).

Significance: Sucrose was higher in green fruit washes and negatively correlated with *Salmonella* growth in washes. Glucose was higher in ripe fruit and positively correlated with growth. These data suggest sugar fluctuations during ripening may modulate *Salmonella* dynamics on fruit surfaces.

P3-119 Modelling *Salmonella* Newport Growth Dynamics in Tomato Fruit Washes Reveals Cultivar and Fruit Ripeness Effects

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Introduction: Fruit ripening is a complex process that leads to changes in tomato surface metabolites. *Salmonella enterica* is efficient in utilizing some of these metabolites for growth. How the shift in tomato surface metabolites during ripening affect *Salmonella* growth or persistence on fruit is not fully investigated.

Purpose: To gain an understanding of how fruit surface metabolites effect *Salmonella* dynamics on tomato, we assessed and modelled *Salmonella* growth in tomato fruit washes as a factor of fruit ripening in various tomato cultivars.

Methods: Tomato cultivars 'Purple Bumblebee', 'Amish Paste', 'Heinz-1706', 'Black Icicle', and 'Emerald Evergreen' were grown in high tunnels in Maryland. Fruit of each cultivar were harvested at a mature green or red ripe stage and washed in sterile water for 90 min to collect surface metabolites. Fruit washes were filter-sterilized and inoculated with ~2.0 log CFU/ml of *Salmonella* Newport. Growth curves were developed by measuring log CFU/mL at 7 different timepoints. Microbial data were fitted to the Roberts and Baranyi Model. Model estimates of lag phase, growth rate and final population value were analyzed using multiple regression.

Results: Both ripeness (p<0.001) and cultivar (p<0.01) were significant factors for *Salmonella* growth in fruit surface washes. *Salmonella* in 'Emerald Evergreen' wash exhibited a longer lag phase than in 'Black Icicle' wash (p<0.05), and a faster growth rate in 'Heinz-1706', 'Black Icicle' and 'Purple Bumblebee' washes (p<0.05). 'Heinz-1706' yielded a higher final *Salmonella* population value (8.1 log CFU/ml, p<0.05) compared to 'Black Icicle' and 'Purple Bumblebee' (7.6 and 7.5 log CFU/ml, respectively). Differences by cultivar were dependent on ripeness stage. Final populations in ripe 'Heinz-1706' washes were signifi-

cantly higher than in green washes of the same (p<0.05) and all other cultivars (p<0.01).

Significance: *Salmonella* growth in fruit surface washes varied among cultivars and fruit ripeness. These data suggest that cultivar differences in fruit surface metabolites can modulate *Salmonella* association on tomato fruit surfaces.

P3-120 Survival of *Salmonella* on Plastic Mulch Treated with Bactericides

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Undergraduate Student Award Entrant

Introduction: Bactericides are used to protect plants from a variety of bacterial plant pathogens. In the pre-harvest environment, *Salmonella* contamination poses a risk to fresh produce. While bactericides target bacterial plant diseases, their effect on foodborne pathogens including *Salmonella*, is relatively unknown.

Purpose: This study investigated the effects of three bactericides possessing different modes of action on the survival of *Salmonella* inoculated on plastic mulch.

Methods: Plastic mulch was cut to fit 100x15mm plates. Four coupons were assigned to six time-points (0, 0.06, 1, 2, 3, and 4d) for each treatment of peroxyacetic acid, copper octanoate, mancozeb, and water. Sterilized coupons (70% ethanol) were spot-inoculated with 0.1mL of a five-strain rifampicin-resistant *Salmonella* cocktail and allowed to air-dry for 90min. Treatments were administered to each dried inoculated coupon at approximately 5 log CFU/cm^2 in the amount of four sprays using a 59mL plastic sprayer. Coupons were placed in a growth chamber (22°C, 55% RH) until each time-point. *Salmonella* was enumerated in duplicate on non-selective and selective media. Coupons below the limit of detection (<0.12 log CFU/cm^2) were enriched in Rappaport Vassiliadis Broth using a modified FDA-BAM Method. Significant differences were analyzed for *Salmonella* concentration by treatment and time-point ($p \leq 0.05$) by Tukey's HSD test in RStudio (V4.2.3).

Results*: Salmonella* concentrations (log CFU/cm^2) were not significantly different between inoculated coupons with no treatment compared to treatment with water across all time-points ($p>0.05$). Significant differences were observed between log CFU/cm^2 of *Salmonella* untreated coupons and water to all bactericide treatments ($p<0.05$). By 4d, remaining *Salmonella* concentrations for each bactericide treatment were 3.66±0.64, 0.80±1.41, and 0.51±0.94 log CFU/cm^2 for mancozeb, copper octanoate, and peroxyacetic acid, respectively.

Significance: Bactericides tested reduced *Salmonella* concentrations on plastic mulch and reduction amount varied by mode of action. Application of bactericides may aid in *Salmonella* management during growing in combination with other mitigations.

P3-121 Impact of Biological Soil Amendments of Animal Origin (BSAAO) on the Persistence of *E. coli* in Florida Soils and Potential to Transfer to Onions: A Two-Year Study

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Introduction: The incorporation of biological soil amendment of animal origin (BSAAO) improves soil health; however, it may introduce and extend the survival of potential foodborne pathogens. This survival is possibly influenced by geographical region, soil type, season, amendment type, and weather conditions.

Purpose: This study evaluated how BSAAOs influenced *E. coli* survival in soil and its transmission to onions during two Florida growing seasons.

Methods: The study (November-April of 2021-2023) utilized twelve 3 m^2 raised-bed plots (n=3/treatment), amended with composted poultry litter (PL) or heat-treated poultry pellets (HTPP) at 680 g/plot, including negative and positive (CE) controls. Soil was spray-inoculated with rifampicin-resistant *E. coli* (10^8 CFU/mL) and samples were collected at 0, 1, 3, 7, 14, 28, 56, 84, 112, 140 days. Enumeration was performed utilizing tryptic soy agar with 80 ppm rifampicin and an MPN procedure utilizing tryptic soy broth with 80 ppm rifampicin when counts were unrecoverable (<0.7 log CFU/g). Onions were harvested 133 days post-transplantation, tested for *E. coli* using an MPN procedure and retested 14 days later after curing.

Results: Survival of *E. coli* was similar (p>0.05) between both years. Amendment type significantly influenced the survival with higher (p<0.05) persistence on HTPP plots than PL and CE. By day 28, *E. coli* rapidly declined and reduced by 4.70±0.82 log CFU/g in CE, 3.80±1.52 log CFU/g in PL but 2.52±0.94 log CFU/g in HTPP plots. In both years, *E. coli* was recoverable from amended plots until day 140 with higher recovery from HTPP (1.49 log CFU/g) than PL (0.76 log CFU/g) plots. Onion samples from HTPP plots were positive for *E. coli* before (14/30) and after (9/30) curing.

Significance: BSAAO, particularly, HTPP has potential to cause extended survival (>140 days) of *E. coli* in amended soil and transfer to crops. Understanding the underlying cause and effective mitigation measures is needed.

P3-122 Survival of *Salmonella* Typhimurium in Poultry Manure Amended Soil and Transference to Tomatoes (*Solanum lycopersicum* 'Micro-Tom')

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Introduction: Outbreaks of foodborne illness are reported every year and many of them are linked to the consumption of fresh produce, including tomatoes. Tomatoes are vulnerable to contamination from several sources, including irrigation water, soil, wild animal feces, and application of manure as fertilizer. Preharvest interventions are crucial to control pathogenic bacteria, thus prompting research on the effect of agricultural practices that can lead to the contamination of fresh produce with foodborne pathogens.

Purpose: Determine the survival of *Salmonella* Typhimurium in soil amended using poultry manure and to compare it with a commercial organic fertilizer.

Methods: One hundred and twenty pots with amended potting soil (poultry manure, n=45, commercial fertilizer, n=45, control, n=30) were used to grow seedlings of tomato var. Micro-Tom under greenhouse conditions. Inoculated amended potting soil was used to assess the transference of *S.* Typhimurium with kanamycin resistance to the above ground parts of the plants and fruits over a 70-day period post inoculation. Analyses were conducted at nine time points (0, 7, 14, 21, 28, 42, 49, 56 and 70 days after inoculation) that determined *S.* Typhimurium persistence in soil up to 70 days by plating on XLT-4 + kanamycin (50 µg/ml). Data were analyzed using one-way ANOVA (p<0.05).

Results: Poultry manure resulted in significantly less survival in soil compared with commercial fertilizer at day 70 (p= 0.0164). The presence of *S.* Typhimurium on tomato plants was > 1 log during the first 14 days on both treatments, a factor that can be attributed to plant size and contact with contaminated soil. Finally, no *S.* Typhimurium was detected in tomato fruits.

Significance: These findings provide insights into controllable agricultural practices that may lead to the reduction of human pathogenic bacteria on fresh produce.

P3-123 Examination of the Persistence of *Escherichia coli* and Protozoan Parasites on Plant Tissue in Soil

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❖ ***Developing Scientist Entrant***

Introduction: Soil is a reservoir for protozoal and bacterial pathogens. Understanding the relationships between these organisms and plant degradation is critical to advancing pre-harvest food safety.

Purpose: Assess the survival dynamics of *C. parvum, E. tenella* (a surrogate for *Cyclospora cayetanensis*), and *E. coli* in soil following their introduction onto plant tissue.

Methods: Collected soil was sent to a soil testing lab for chemical analysis and then distributed into 7oz standup sampling bags (approximately 120g/bag, n=130 bags). Fresh basil and cilantro were procured from a local distributor with 6g weighed into individual petri dishes (n=65/plant type). *C. parvum* and *E. tenella* oocysts (10^6 oocysts in 100µl each), of which greater than 90% were sporulated, and *E. coli* TVS355 (10^7 CFU in 100µL), were then inoculated dropwise onto each plant sample and airdried for 120min. One plant sample was then added to each soil bag and massaged by hand until the tissue was at a depth of 17cm. Samples were incubated at 12 or 32°C. Periodically over the course of 6 months samples were removed from incubation and assessed. *E. coli* survival was assessed through traditional enumeration on MacConkey agar, while infectivity of parasitic targets was assessed via mammalian cell culture coupled with qPCR. Statistical analysis was performed using JMP via ANOVA.

Results: After 30-days of incubation no significant (p>0.05) reduction in *E. coli* titer was measured, this result is consistent across both experimental temperatures and produce types. The infectivity of protozoan parasites was not significantly (p>0.05) reduced after 30-days, regardless of plant type and incubation temperature. The greatest reductions were observed in *E. tenella* on cilantro incubated at 32°C, decreasing by 0.2 log infectious oocysts after 30-days.

Significance: This is the first study of its type assessing bacteria and protozoa persistence from decaying plant and soil matrices, emphasizing the need for practical and comprehensive soil management and risk mitigation strategies to address contamination.

P3-124 Organic Fertilizers Support Survival of Pathogenic and Non-Pathogenic *Escherichia coli* in Soils and Sporadic Transfer to Romaine Lettuce

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Introduction: Biological soil amendments are an essential input in organic lettuce production. However, these organic fertilizers can introduce or transfer pathogens like *Escherichia coli* O157:H7 to lettuce in pre-harvest environments.

Purpose: To evaluate the effect of organic fertilizers on survival of non-pathogenic and pathogenic *E. coli* in soils and transfer to lettuce.

Methods: Romaine lettuce was grown with controlled light, temperature, and relative humidity. Soil was amended (side-dressed) with either heat treated poultry pellets (HTPP), HTPP with corn steep liquor (CSL), seabird guano (SBG), SBG with CSL, or left unamended (UA). Soils were co-inoculated with non-pathogenic, rifampicin-resistant *E. coli* TVS 353 and two chloramphenicol-resistant *E. coli* O157:H7 isolates (100 mL of 10^6 CFU/mL). *E. coli* survival over 28 days was evaluated. On day 28, Romaine lettuce was harvested, and presence of *E. coli* was determined. *E. coli* survival data were fitted to four models (log-linear, Weibull, Biphasic, Geeraerd), with the best fit model selected based on Akaike Information Criterion.

Results: Levels of *E. coli* on day 0 were ca. 5 log CFU/g soil. By day 28, all treated soils contained less than ca. 2 log CFU/g except one treatment (*E. coli* O157:H7 in HTPP+CSL). On Day 28, 13.3% (6/45) and 11.1% (5/45) of lettuce plants contained *E. coli* TVS 353 and O157:H7, respectively. The log-linear model best-fit *E. coli* TVS 353 survivals in soils. For *E. coli* O157:H7, Weibull was the best-fitting model for SBG, log-linear was the best fitting model for HTPP and UA, and biphasic was the best fitting model for HTPP+CSL.

Significance: *E. coli* survived but declined in soils containing organic fertilizers over 28 days. Transfer of *E. coli* from soils to Romaine lettuce was sporadic and could not be measured quantitatively. Our data show treated biological amendments used in organic lettuce production do not enhance survival of *E. coli* in soils.

P3-125 Survival of Lactic Acid Bacteria in Poultry Litter Systems from Commercial Operations

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Introduction: Application of lactic acid bacteria (LAB) as a probiotic has been gradually increasing in animal production to reduce pathogen contamination of animal-sourced food. Probicon, a novel, new probiotic, has been evaluated for successful use in cattle systems, but to date, hasn't been evaluated in poultry operations.

Purpose*:* Determine the survival of Probicon, a commercially available lactic acid bacteria, after application to poultry litter collected from commercial operations.

Methods*:* Litter samples were taken on week one, four, and seven of the growth process from commercial broiler chicken houses. Samples were mist-inoculated according to four treatments: negative control (water), LAB only, *Salmonella* only, and LAB and *Salmonella*. Cocktails of *Salmonella* were made at a concentration of 1 x 10^9 CFU/g with a target concentration of 1 x 10^6 CFU/g. Litter samples were incubated for seven weeks at 37°C, with 10g of sample being removed once a week for LAB quantification with MRS plates using spread plating methodology and for *Salmonella* detection using the Hygiena RT-PCR BAX system.

Results*:* Lactic acid bacteria counts began at an average concentration of 4.1 log CFU/sample on week one and presented a gradual decrease in the consecutive weeks with values of 3.26 log CFU/sample, 3.06 log CFU/sample, 2.04 log CFU/sample, 1.98 log CFU/sample, and 1.70 log CFU/sample, until they reached 0.0 log CFU/sample in week seven. A Kruskal-Wallis test was performed with no significant difference among treatments (p=0.669). The treatments that were inoculated with LAB did provide higher overall total counts. There were no *Salmonella* detected in the samples indicating a rapid death in all environments.

Significance: The presence of lactic acid bacteria in poultry litter can improve the microflora of the gut and decrease the bacterial load in poultry products due to their tendency of consuming their own litter. Additional work will be conducted to increase Salmonella in the litter to determine the efficacy of pathogen reduction.

Poster

P3-126 *Salmonella enterica* Contamination of Cucumber Fruit When Introduced to Blossoms Using Aerosolized Poultry Litter Particulates

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Introduction: *Salmonella* outbreaks have been associated with consumption of fresh cucumbers. Adjacent animal operations may contribute to the introduction of *Salmonella* onto crop plants.

Purpose: To investigate the ability of *Salmonella* to colonize and internalize cucumber fruit when applied to blossoms via contaminated poultry litter at varying inoculum levels.

Methods: Cucumber plants (*Cucumis sativus* var. *sativus*) cultivar Marketmore 76 (slicer) were grown from commercial seed and maintained in a BSL-3P phytotron greenhouse. *Salmonella* Poona was incorporated into sterilized commercial poultry litter as a freeze-dried pellet and introduced via blossoms at two inoculum levels (ca. 5.6 or 4.4 $\log_{10}$ CFU/blossom) via aerosolized contaminated poultry litter (ca. 10 mg applied to each blossom). In total, 72 Marketmore 76 plants at the blossom stage were divided into three treatment groups: a negative control group [dosed with untreated poultry litter (skim milk); n=16] and two treatment groups [inoculated with ca. 5.6 $\log_{10}$ CFU/blossom (n=28) or ca. 4.4 $\log_{10}$ CFU/blossom (n=28)]. Cucumbers (harvested 4-32 days post inoculation; average weight 261.8 g) were analyzed for *Salmonella* by enrichment in accordance with modified FDA-BAM methods. Data were analyzed for prevalence of contamination (surface and inside), and the Pearson Chi-Square Fisher's Exact test was used to determine significant differences in sample positivity (i.e., fruit colonization) obtained for inoculated fruit over inoculum levels.

Results: Of the total mature fruit harvested from ca. 5.6 $\log_{10}$ CFU/ml (n=91) or 4.4 $\log_{10}$ CFU/ml (n=64), 60.4% (55/91) or 26.6% (17/64) were contaminated and 13.2% (12/91) or 3.1% (2/64) had *Salmonella* internalized into the fruit, respectively. Both surface (X^2=17.336, p<0.001) and internal (X^2=4.630, p=0.0266) contamination were significantly lower when applied at the reduced inoculum level.

Significance: These results from a controlled growing environment identified poultry litter as a potential vehicle for *Salmonella* to contaminate cucumbers when introduced to blossoms during pre-harvest.

P3-127 Poultry Litter Particulates as a Vehicle for *Escherichia coli* O157:H7 Contamination of Romaine Following Freeze Damage

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Introduction: Romaine lettuce has been implicated in recurring outbreaks associated with shiga-toxin producing *Escherichia coli*. Animal operations adjacent to crop production may contribute to O157 contamination.

Purpose: To examine the potential contamination risk to freeze damaged tissues of romaine lettuce by O157 via aerosolized contaminated poultry litter to mimic contaminated fugitive dust.

Methods: Lettuce (*Lactuca sativa* L.; cultivar 'Green Towers') plants (n=60) were grown from commercial seed and maintained in an environmental chamber at 60% relative humidity, 20°C/10°C day/night temperature and 12 h photoperiod. At 78 d post planting, plants were exposed to -10 °C for 10 min (freeze damage; n=30) or unexposed to freeze (no damage; n=30). Each set of 30 plants were subdivided into two treatment groups: a negative control group [n=10; dosed with untreated poultry litter] and a treatment group [n=20; inoculated with ca. 5.9 log CFU/leaf]. At 7 dpi (days post inoculation), leaf samples were enumerated and plated on ChromAgar O157. Aliquots from leaf enumerations and heads were processed and enriched for O157 in accordance with FDA BAM methods. Data were analyzed for prevalence of contamination and the Pearson Chi-Square Fisher's Exact test was used to determine significant differences in sample positivity.

Results: For enriched samples harvested from O157-inoculated plants, 55.0% (11/20) leaves and 80.0% (16/20) heads freeze damaged and 30.0% (6/20) leaves and 75.0% (15/20) heads undamaged were found to harbor O157. Prevalence of contamination was not statistically significant when comparing freeze damage to no damage for both leaves (X^2=2.558, p=0.2003) and heads (X^2=0.143, p=0.7050). Between ca. 1.7 and 4.5 $\log_{10}$ CFU *E. coli* O157/leaf was recovered from positive leaf samples 7 dpi.

Significance: Results from this environmental chamber study demonstrated that poultry litter as a mimic for fugitive dust could contribute to romaine lettuce contamination 7 d prior to harvest. Future studies should examine level of low temperature-induced tissue damage and O157 persistence.

P3-128 Impact of Environmental Conditions on the Survival of *Cryptosporidium parvum* Oocysts on Soil and Manure

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Introduction: *Cryptosporidium parvum*, a protozoan parasite, is a serious threat to public health and agriculture. Risk assessment and management require an understanding of its persistence in soil and manure.

Purpose: The purpose of this study was to quantify and compare the die-off rates of *Cryptosporidium parvum* oocysts in soil and manure at regulated temperatures and humidity levels.

Methods: One g of field soil and 1.75 g fresh cow manure were mixed and inoculated with 100 µl of 10^6*Cryptosporidium parvum* oocysts. Samples were incubated in a growth chamber in a combination of temperatures ranging from 1°C to 18°C and relative humidity between 50% to 75% to imitate the Baton Rouge, weather conditions for December-January. Growth chamber was set to a diurnal cycle of light and dark phases over 30 days. Oocyst viability was assessed at predetermined intervals (Day 0, 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 30) using fluorescence microscopy. A first-order decay model was applied to determine the die-off rates, and ANOVA for viability assessment.

Results: The die-off rate of *Cryptosporidium parvum* oocysts in soil was significantly higher (p<0.005) (-0.0035 per day) compared to manure (-0.0018 per day). The viability of Cryptosporidium oocysts in the soil gradually declined with notable variations (p<0.05) in viability rates between day 0 and days 24, 27, and 30. Comparably, oocyst viability in manure decreased significantly, especially after two and a half weeks, with significant differences (p<0.05) seen from day 0 to days 18, 21, 24, 27, and 30.

Significance: In the specified environmental settings, the die-off rate of Cryptosporidium oocysts was found to be faster in soil than in manure. The study's findings implies that conditions in manure tend to be less favorable to oocyst persistence as those in soil, which may have implication for public health and agricultural operations.

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P3-129 Influence of Crop Species and Compost on the Soil Microbiome over Time

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Introduction: The soil microbiome plays an important role in human and plant pathogen interactions and is a critical component of One Health.
Purpose: Determine the impact of crop species and compost on the soil microbiome diversity over time.
Methods: Compost and soil were mixed to create 8 separate plots in a high tunnel in Beltsville, MD. Dill and fescue were seeded initially, followed by spinach and romaine, and lastly all plots were seeded with romaine only. Every 4-8 weeks, two 1"x6" soil cores were collected/plot along with 2 random samples of crop leaves, when available. After DNA extraction, library preps (Illumina DNA Prep) were sequenced on the Nextseq 2000. Data were analyzed with Kraken Braken and an in-house custom k-mer fungal database. Nonmetric multidimensional scaling and functional profiling using HuMAnN2 analyses were also performed.
Results: Compost had the largest effect on fungal and bacterial composition and was dominated by *Thermobifida, Sphaerobacter,* and *Acytosteliales* spp. A distinct microbiome was found on crop leaves, and it varied between crop species. Crop species had a transient effect on both fungal and bacterial soil microbiome. However, this effect diminished quickly, particularly for the bacterial microbiome. For instance, *Pseudomonas* sp. and *Epicoccum nigrum* were only found on crop leaves and in the soil shortly after crop germination. When plots were rotated into romaine, there was an increase in species with a relative abundance less than 2.5%, which may indicate an increased level of biodiversity. Functional profiling also exhibited dynamic shifts in nucleotide and amino acid biosynthesis pathways in soil microbiome following crop rotations.
Significance: Compost amendment of soil had a greater effect on soil microbiome composition than did crop species in this plot study; however, crop rotation may also play a role in microbial diversity, but additional research is needed.

P3-130 Effect of Using Treated or Untreated Biological Soil Amendments of Animal Origin on the Food Safety Risk of Sweet Potatoes (*Ipomoea batatas*)

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❖ ***Developing Scientist Entrant***

Introduction: Biological Soil Amendments of Animal Origin (BSAAOs) provide essential nutrients to plants but are an important source of food-borne pathogens. Identifying science-based application techniques and treatments to minimize contamination will promote the safe use of BSAAOs.
Purpose: This study evaluated the effect of using composted or raw manure soil amendments on the quality and safety of sweet potatoes (variety *Orleans*).
Methods: The field trial was located at the LSU AgCenter Botanical Gardens, with a split-plot experimental design, having 36 plots, each measuring 60 ft^2. The treatments included composted cow manure (CM), raw manure (RM), or no-BSAAO application (NB), with each treatment applied either through tilling or no-tilling. Soil samples were collected during weeks 1, 3, 5, 10, and 15 and sweet potatoes were harvested on weeks 17, and 18, two days after using irrigation water contaminated with Nalidixic acid-resistant *E. coli* (6 log CFU/mL).
Results: *E. coli* levels in soil from CM plots tilled and no-till, were highest after weeks 10 (1.32 log CFU/g) and 15 (1.30 log CFU/g). In RM plots *E. coli* levels were significantly higher after application in week 1 (1.44 log CFU/g) and in week 3 (1.59 log CFU/g). Plots with RM no-till had the highest *E. coli* levels during weeks 1 (1.51 log CFU/g), 3 (1.59 log CFU/g dry weight), and 15 (1.42 log CFU/g). *E. coli* (0.29-0.50 log CFU/cm^2) and *Coliforms* (0.86- 0.95 log CFU/cm^2) were also higher on sweet potatoes from RM plots irrigated with contaminated water. The treatments influenced the quality of sweet potatoes, the amount of marketable (3.8505-4.7510 kg/m^2) sweet potatoes was significantly higher than non-marketable (0.2430-0.5500 kg/m^2) sweet potatoes (p<0.001*).*
Significance: *E. coli* can persist in no-till RM treatments throughout the sweet potato crop cycle. The *Orleans* sweet potato variety produces high-yield marketable sweet potatoes regardless of the applied treatments.

P3-131 Effect of Biochar on Generic and Antibiotic-Resistant Bacteria in Dairy Cattle Manure Composting

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Introduction: Biochar dairy manure amendment may enhance composting effectiveness as a preharvest food safety measure to reduce foodborne bacteria.
Purpose: We evaluated the effect of composting dairy manure amended with four levels of biochar treatments on the concentrations and prevalence of generic (*E. coli* and enterococci), and antibiotic resistant bacteria (extended spectrum beta-lactamase producing (ESBLs) *E. coli*, 3rd generation cephalosporin resistant [$3GC^r$]- and tetracycline resistant [TET^r]-*E. coli*, and TET^r enterococci).
Methods: Compost starter mixture, prepared from equal amounts of fresh dairy manure and saw dust, was mixed with 0%, 2%, 4%, or 10% (w/w) biochar. The mixture was distributed into 16 (4/biochar treatment) plastic rotary drum reactors. Composting was run for six months. Samples collected from the manure mix (n=16) and from the final compost (n=16) were cultured for the enumeration of the bacterial strains. Data were analyzed by Poisson regression model to assess the effects of biochar concentrations and composting phase (manure and compost) on bacterial concentrations.
Results: Composting significantly reduced generic *E. coli* concentrations in a dose-response manner (p=0.02); the highest effect observed in 10% biochar. Composting also significantly (p=0.015) reduced the concentration of generic enterococci, with no significant (p=0.304) dose-response relationship with respect to percent biochar. TET^r *E. coli* were not enumerable from compost samples and TET^r enterococci were enumerable only from a single compost sample obtained from 0% biochar. $3GC^r$-and ESBLs-*E. coli* were detected from all raw dairy manure mix and not from any compost samples.
Significance: Composting effectively removes antibiotic resistant bacteria, while also significantly reducing concentrations of generic strains from dairy manure. Biochar amendments to dairy manure significantly reduced generic *E. coli* concentration in a dose-response manner. Biochar did not modify the effect of composting on the concentrations and detection of other bacterial strains. Other potential benefits of biochar such as composting duration and quality should be assessed under field conditions.

P3-132 Minimum Concentrations of Slow Pyrolysis Paper and Walnut Hull Cyclone Biochars Required to Inactivate *Salmonella enterica* in Soil

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Introduction: Biochar has been shown to have the ability to inactivate foodborne pathogens in soil. We previously reported that 2.5% of either paper biochar (PB) or walnut hull cyclone biochar (WHB) inactivated >5 log CFU/g of *E. coli* O157:H7 in soil by week 2, based on alkaline pH.
Purpose: A study was conducted to determine the minimum concentrations of PB and WHB required to inactivate *Salmonella* in soil.
Methods: Soil was adjusted to 17.75% moisture and two types of slow pyrolysis biochar (PB, pyrolyzed anoxically at 700°C for 1 h, and WHB [provided by All Power Labs, Berkeley, CA]) were added to soil. Based on preliminary studies, 0.5-2.0% of PB and 2.5-6.5% of WHB, respectively, were mixed into soil. Biochar-amended soil was inoculated with 7.40 log of a two-strain composite of *Salmonella* Newport and Stanley, mixed thoroughly, and stored at 21°C for

6 weeks. Samples were analyzed weekly by plating on TSA, allowing injured cells to recover at 37°C for 2 h, then overlaying with XLT4 agar and incubating at 37°C for another 16 h.

Results: The soil-only positive control samples supported *Salmonella* populations of 7.87-7.56 log from weeks 1-6. *Salmonella* was significantly reduced, in comparison to positive controls (p<0.05), at weeks 1-6, with ≥1.0% PB, in comparison with the required ≥2.5% WHB. While 2.5% WHB inactivated only 3.42 log of *Salmonella* by week 6, 3.5% WHB was required to inactivate ≥5.0 log, which was accomplished by week 1. In contrast, 1.0% PB inactivated 3.14 log by week 6, and only 1.5% PB inactivated >5 log by week 2. Based on these results, only 1.5% PB is needed to reduce >5.0 log of *Salmonella* in soil, while ≥3.5% WHB is required to achieve the same results.

Significance: These results may provide guidance on application of PB and WHB to crop soil for inactivating *Salmonella enterica*.

P3-133 Evaluation of Pecan Extract Priming and Coating for *Listeria monocytogenes* Decontamination in Lettuce Seeds

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❖ ***Developing Scientist Entrant***

Introduction: Contaminated seeds can present a significant food safety risk in a controlled environmental agricultural (CEA) production. Seeds treated with natural bioactive compounds may actively control the growth of pathogens when contaminated.

Purpose: This study investigated the application of pecan extract on seeds to control the growth of *Listeria monocytogenes* and its effect on germination.

Methods: Salad lettuce bowl green (0.25 g) seeds were inoculated (2.5mL) at 8 log CFU/g with a cocktail of *Listeria monocytogenes* (101M, V7, LCDC and Scott A) and were treated with pecan extract. For coating 5 and 10% of aqueous pecan extract with sodium alginate (2 and 4%) was used. The samples were diluted in 1XPBS solution and enumerated on Oxford agar base after incubation (37°C-24h). Effect on germination after treatment was also evaluated. Lettuce seeds (n=100) were expose to each treatment combination prior to germination and number of germinated seeds was measured every day during 7-day period. All experiments were conducted in triplicate. Data were analyzed by SAS9.4, ANOVA using and LSMeans were applied to determine any significant differences (p<0.05).

Results: Priming treatments reduced *Listeria monocytogenes* on seeds by 2.03 to 3 log CFU/g with a significant reduction of 3±1.17 log CFU/g (p <0.05) with 10% of pecan extract for 6 hours. Increasing the priming time had no effect on reduction of *Listeria*. Coating treatment reduced *Listeria* between 0.42 to 1.06 log CFU/g. A significant (p<0.05) reduction of 1.06 ± 0.43 Log CFU/g was achieved with the lowest concentration of pecan extract (5% of pecan extract and 2% of alginate). However, *Listeria* levels remained similar even after increasing the concentration of pecan extract to 10%. The coating and priming treatments had no effect on the germination properties of the seeds.

Significance: Application of pecan extract on seeds by priming or coating could control the growth of *Listeria monocytogenes* in contaminated seeds.

P3-134 The Efficacy of Drytec® and Tsunami®100 Treatments in Inactivating Enterohemorrhagic *Escherichia coli* on Alfalfa Seeds and Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: The sprout industry faces an ongoing challenge in managing recurring outbreaks linked to the consumption of contaminated alfalfa sprouts, demanding effective control of pathogenic bacteria on alfalfa seeds.

Purpose: This study examined the efficacy of treatments with Drytec® (calcium hypochlorite) and Tsunami®100 (peroxyacetic acid) in inactivating enterohemorrhagic *E. coli* (EHEC) on alfalfa seeds and sprouts.

Methods: Four individual EHEC cell suspensions (*ca*. 5 log CFU/g) were used to inoculate alfalfa seeds (2 g) at room temperature for 1 h. Inoculated seeds were rinsed with sterile water, and then treated with Drytec® for 15 min or Tsunami®100 for 30 min, and control seeds underwent sterile water treatment for 15 and 30 min, respectively. Treated seeds were sprouted on 1% water agar at 25°C for 7 days, with periodic assessments of EHEC populations. Data were fit into the general linear model and analyzed using ANOVA. Fisher's least significant test was used to separate the means at a 95% confidence interval.

Results: The mean EHEC inoculation level on alfalfa seeds was 2.63 log CFU/g. No EHEC cells were detected following seed treatments with Drytec® and Tsunami®100. During sprouting, Drytec®-treated seeds had no detectable EHEC even from the enrichment assay, while at some sampling points, Tsunami®100-treated seeds had a mean recovery of 0.80 log CFU/g or less of EHEC cells. Treatments with Drytec® and Tsunami®100 reduced mean EHEC populations on sprouts by 4.54-4.60 log CFU/g and 1.52-1.25 log CFU/g, respectively, compared to the water treatment controls. Mean populations of the four strains differed significantly (p<0.05), with *E. coli* K4492 (*E. coli* O157:H4) having the highest and *E. coli* BAA-2326 (*E. coli* O104:H4) having the lowest mean cell populations on sprouts. EHEC cell population increased with sprouting time until Day 3.

Significance: Drytec® and Tsunami®100 treatments demonstrate effectiveness in suppressing the growth of EHEC on alfalfa seeds and sprouts.

P3-135 Integrated Crop-Livestock Farming Influences the Incidence of Foodborne Pathogens and Indicator Bacteria on Fresh Local Produce in Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Integrated crop-livestock farms (ICLFs) utilize biological soil amendments of animal origin (BSAAOs) to maintain/improve soil fertility/health. However, BSAAOs may introduce pathogens into soils that can contaminate fresh produce due to error in decomposting practices.

Purpose: To investigate impacts of ICLF practices on the ecology/transmission of foodborne pathogens and indicator organisms to fresh produce by comparing similar samples from crop-only farms (COFs), farmer's-markets (FMs), and retail-stores (RTs).

Methods: Altogether, 1,782 samples comprising soil, animal reservoir, water, and produce from ICLFs, COFs, FMs, and RTs were analyzed following standard protocols. Aerobic bacteria (APC) and generic *Escherichia coli* (g*EC*) were enumerated using petrifilms, while *Salmonella, Listeria monocytogenes* (*Lm*), and Shiga toxin-producing *Escherichia coli* (STEC) and virulence factors (VFs) were detected by culturing with PCR confirmation. ICLF-soil fertility/health was evaluated pre- and post-BSAAO incorporation.

Results: BSAAO incorporation improved soil fertility/health parameters such as phosphorous, potassium, calcium, and other properties important to crop growth. Detection of *Salmonella*, *Lm*, and STEC/VF-genes was increased in ICLF soils compared to COF-soils. ICLF-soils were significantly (p<0.05) more positive (2.72%) for *Lm* than COF-soils (0%). On ICLFs, higher overall pathogen prevalence and mean log APC, g*EC*, and total coliform levels were associated with animal pen and manure/compost samples. ICLF-produce had increased pathogen prevalence with 0.39%, 1.95%, and 13.62% of samples positive for *Salmonella*, *Lm*, and STEC/VF-genes, respectively. In COF-produce samples *Salmonella* and *Lm* went undetected and 5.33% were positive for STEC/VF-genes. *Salmonella* and *Lm* were each detected in 0.43% of FM-produce, and STEC/VF-genes in 0.37% of RT-produce. Amongst all samples there was a statistically

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significant (p<0.05) positive relationship between total coliform levels and pathogen prevalence. The STEC/VF-genes O103 and *stx*2 were most frequently isolated, while Bareilly was the leading *Salmonella* serovar.

Significance: Foodborne pathogen prevalence associated with BSAAOs and produce samples from farming and retail settings highlights contamination risks associated with fresh produce.

P3-136 Microbiological Assessment of Foodborne Pathogens in Farmers' Markets on the Eastern Shore of Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Farmer's markets (FMs) benefit from consumer perceptions and are a source of local fresh produce. Over the past two decades, the number of FMs have significantly increased and have been accompanied by a positive relationship with the number of reported foodborne illnesses. Compared to traditional retail counterparts, FM products tend to be less stringently regulated and receive minimal/no sanitization processing, which may increase the opportunity for contamination by foodborne pathogens.

Purpose: In this study, we assessed the microbiological contamination of produce from Maryland Eastern Shore FMs.

Methods: In total, 626 produce samples were obtained from five FMs over a two-year period. Total aerobic bacteria (APC) and generic *Escherichia coli* were enumerated by petrifilms; *Salmonella* and *Listeria monocytogenes* were detected using standard culture and PCR confirmation methods.

Results: Approximately 1% (6/626) of produce samples were positive for at least one tested pathogen and 3.51% (22/626) were positive for *E. coli*. Specifically, 0.64% (4/626) and 0.32% (2/626) of samples were positive for *Salmonella* and *L. monocytogenes*, respectively. A majority of these detections occurred during summer with *Salmonella*, *L. monocytogenes*, and *E. coli* being isolated from 75% (3/4), 100% (2/2), and 63.64% (14/22) of positive samples during the summer, respectively. Produce APC values ranged from 1.60 log CFU/g–8.16 log CFU/g, *gEC* from <1 log CFU/g–4.30 log CFU/g, and total coliforms from <1–log 5.80 CFU/g. Average APC (6.22 log CFU/g) levels in summer were significantly greater (p<0.05) than fall and spring levels, while total coliform (3.34 log CFU/g) levels were significantly greater (p<0.05) in summer compared to fall.

Significance: This study validated contamination hazards associated with fresh produce available at some of the FMs on the Maryland Eastern Shore. Continued research/education regarding good agricultural and handling practices by producers and consumers are crucial to reducing produce associated foodborne illnesses.

P3-137 Managing Adjacent Land-Use Risks Associated with *Salmonella* and Shiga-Toxin Producing *E. coli (STEC)* from U.S. Cattle Operations

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Introduction: Proximate livestock operations have been suspected as the source of pathogens in recent produce outbreaks, However, there is a lack of data with respect to quantifying populations of foodborne pathogens in bioaerosols, specifically *Salmonella* and *Shiga-toxin producing E. Coli (STEC)*, and their potential spread to nearby areas to determine what interventions may be appropriate.

Purpose: The purpose of this study is to assess the risks associated with aerosol transmission of *Salmonella* and *STEC* produced in Southeastern cattle operations.

Methods: Sampling was conducted at three different cattle operations during the typical production window for fresh produce, ranging from November to May 2024. Anderson impact samplers consisting of 2 stages each, were used at three different heights (0.5 m, 2 m, 5 m). Samples were collected at 0 m, 50 m, 100 m, 400 m, and 650 m- 1000m from the animal operation boarder for 10 minutes at each distance. Chromagenic selective agars were used to isolate *Salmonella and STEC.* A weather station was used simultaneously with the Anderson impact samplers, where relative humidity, temperature and wind direction were analyzed. Samples were incubated after sampling at a temperature of 35°C for 48 hours and enumerated.

Results: Temperature and relative humidity ranged from 5.8-36.9 °C and 21-81% respectively. Maximum wind speeds ranged from 6.4-16.0 m/s, respectively. Presumptive *Salmonella* isolates and *STEC* were detected up to 1,000m. Suspected *Salmonella* colonies were found in 80/336 samples, followed by STEC with a total of 10/336 suspected samples.

Significance: Understanding the extent to which *Salmonella* and *STEC* may be transmitted to adjacent lands will help identify appropriate mitigation strategies.

P3-138 Understanding the Potential for Bioaerosol Contamination from Cattle Operations on Adjacent Land

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Introduction: Bioaerosol contamination from animal operations on adjacent land has been identified as a potential risk to produce. There is little data quantifying populations of potential foodborne pathogens in bioaerosols to assess this risk or develop mitigation strategies.

Purpose: This study aimed to quantify risks tied to bioaerosols from cattle production in Florida and Georgia.

Methods: Air samples were collected for 10 min downwind of three cattle operations using 2-stage impact samplers (28.31 L/min). Sampling sites were 0, 50, 100, 400, and 650-1000 m from the animal operation perimeter and at 0.5, 2, and 5 m heights. Chromogenic media was used to isolate coliforms and generic *E. coli*. For each sampling event, meteorological data was collected from weather stations mounted next to the impact samplers. Samples were incubated at 35 °C for 48 h before enumeration; the limit of detection is 0.49 log CFU/m^3.

Results: At locations 1, 2, and 3, air temperature and relative humidity ranged from 5.8 – 34.8, 8.9 – 26.2 and 16.8 – 36.9 °C, and 32 – 86, 32 – 83 and 21 – 81%, respectively. Maximum wind speeds were 6.4, 7.0, and 16.0 m/s, respectively. Coliforms were more frequently detected than generic *E. coli* across all sites, distances, and locations. Coliforms were present in 74/336 samples with levels ranging from 0.55 – 2.09 log CFU/m^3. The frequency of coliform detection varied by site (22/96, 19/90, and 33/150, respectively), distance (13/72, 36/72, 11/72, 18/72, and 9/48, respectively), and height (30/112, 25/112, and 19/112, respectively). Generic *E. coli* was detected in 17/336 samples with levels ranging from 0.55 – 1.03 log CFU/m^3. Generic *E. coli* was not detected beyond 400 m.

Significance: Low levels of coliforms and generic *E. coli* were detected, with the frequency generally decreasing as distance increased.

P3-139 Monitoring of Viral Contamination in Agricultural Settings: A study of Fresh Produce, Irrigation Water, and Soil in South Korea

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Introduction: Norovirus (NoV) is the primary cause of acute gastroenteritis, often linked to contaminated water and food, posing a significant public health concern. With the growing demand for fresh produce due to increasing health awareness, understanding NoV contamination in agriculture, particularly in soil and irrigation water, is crucial.

Poster

Purpose: This research focuses on evaluating the presence of viral hazards in fresh produce, irrigation water, and soil within agricultural contexts.
Method: In this study, 39 fresh produce samples, 18 irrigation water samples, and 39 soil samples were collected from various locations across South Korea. These samples were processed according to the "Foodborne Pathogen Investigation Test Methods" by the Korea Ministry of Food and Drug Safety. Detection of norovirus GI/GII was conducted using RT-qPCR, and crAssphage, a marker for human fecal contamination, was identified using qPCR. Additionally, 16S rRNA amplicon sequencing was employed to observe differences in microbial diversity between NoV positive and negative samples.
Results: Out of 96 samples, 29 (30.2%) tested positive for norovirus (GI and GII), with specific detection in water (12/18), soil (9/39), and fresh produce (8/39). CrAssphage was found in 22 samples (22.9%), evenly distributed between agricultural water and soil. Co-occurrence of norovirus and crAssphage was noted in 11 samples (11.4%). Microbial community analysis showed Proteobacteria dominance at the Phylum level in both NoV positive (36.4%) and negative (39.8%) samples. In the family level, Moraxellaceae (15%) was prevalent in NoV positive samples, while Pseudomonadaceae (22%) and Comamonadaceae (24%) were dominant in negative samples. At the Genus level, *Acinetobacter* (21.1%), *Pseudomonas* (16.1%), and *Gemmatimonas* (14%) were predominant in positive samples, with *Pseudomonas* (30.5%) and *Limnohabitans* (16.8%) leading in negative samples.
Significance: This study highlights the nature of viral contamination in agricultural settings in South Korea, emphasizing the need for monitoring and control measures.

P3-140 Efficacy and Impact of Sanitizers in Controlling Pathogenic Bacteria on Irrigation Water and their Impact on Quality and Safety of Romaine Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: Romaine lettuce (RL) has been linked to 324 outbreaks from 2013-2021, often associated with pre-harvest farming practices and contaminated irrigation water (2018-2022). Managing water sources and treatments is crucial for consumer safety. The main challenges in using sanitizer-treatments for irrigation water include efficacy, impact on plant and consumer safety, and effects on soil-health and crop productivity.
Purpose: To evaluate the effectiveness of three sanitizers (sodium hypochlorite-Chlorine-60ppm, peroxyacetic acid-PAA-30ppm, sodium bisulfate monohydrate-SAS-0.60%) plus a water-control, on the inactivation of pathogenic bacteria in RL and their impact on soil and plant health.
Methods: Two RL varieties (red-cv.Ezbruke/green-cv.Costal Star) were grown for 10-weeks. Plants were spray-inoculated with a 50ml bacterial cocktail (*Listeria monocytogenes*-LM, generic *E. coli*-W9, *E. coli* O157:H7-EC) at log 5-CFU/mL. After 24-h of inoculation; 50ml of each sanitizer/water was sprayed on each plant. Sanitizer concentrations met EPA's 3-log reduction requirements. Bacterial recovery was performed at 0-, 4-, and 8-days post-inoculation using selective-differential media (CHROMagar™-Listeria/Rif50-LM, and CHROMagar-O157/Rif50-EC). The experiment was replicated twice. Probe-based PCR was performed to determine the presence of the inoculated strains. Evaluated soil-health parameters (pH, cation exchange capacity-CEC, soluble salts-EC, soil elements). Statistical evaluations; (1)non-parametric analysis Kruskal-Wallis and (2)ANOVA, Tukey-Test; R-studio.
Results: Chlorine, SAS, and PAA showed a 0.5-log reduction when compared to water for all inoculated strains (p<0.05). LM was more prevalent in lettuce regardless of sanitizer-treatment. Lettuce variety did not impact bacterial survival (p>0.05). Soil-health parameters were identical between sanitizers-treatments; however, soil-EC was significantly impacted by EC variations ranging from 1.3 to 5.2mmho/cm across treatments. SAS and PAA had higher sodium content (4.17 and 3ppm) compared to water and chlorine treatments (1.67 and 1.83ppm) (p<0.05).
Significance: Sanitizer-solutions have marginal effects on pathogen inactivation on the surface of RL. All three sanitizers similarly impacted soil-health, despite their different formulations. Soil EC was significantly affected by all sanitizers, which could reduce plant productivity if not managed properly.

P3-141 Evaluating the Efficacy and Impact of Sanitizers in Controlling Pathogenic Bacteria on Irrigation Water Based on EPA Standards

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❖ ***Developing Scientist Entrant***

Introduction: Irrigation water quality is vital in fresh produce. The lack of practical microbial water standards and EPA-registered sanitizers for controlling human pathogens in irrigation water challenges growers, affecting food security.
Purpose: To compare four commercial sanitizers (sodium hypochlorite-Chlo, peroxyacetic acid-PAA, sodium acid sulfate-SAS, potassium acid sulfate-KAS) at different concentrations and water chemistries for a 3-log reduction of STEC, Salmonella-SM, or Listeria monocytogenes-LM and their corrosive effects on aluminum and stainless-steel.
Methods: Sanitizer efficacy was tested under varying turbidities (0, 50, 100, 150NTU), contact times (CT; 3-5min), pH, and concentrations (SAS-KAS, 0.4%-0.6%; Chlo, 15-60ppm; PAA, 10-30ppm). In sterile cups, 100ml water with specific turbidity was inoculated with STEC, SM, or LM at log 6 and 3cfu/mL. After each CT, all sanitizers were neutralized before bacterial enumeration. Corrosion capacity was tested in two stages each 14-days in length and at 23-73℃ (Stainless-steel (SS304-SS316); Aluminum (AL3003-3004)). Coupons were submerged in sanitizer solutions and corrosion rates were determined by the weight difference before and after each incubation phase. Statistical evaluation non-parametric analysis Kruskal-Wallis, R-studio.
Results: Bacterial inactivation has a negative correlation (-0.086) with turbidity for Chlo, PAA, but not SAS (p<0.05). CT significantly impacted inactivation for all sanitizers (p<0.05). SM and STEC showed higher inactivation than LM (p<0.05). Chlo at 60ppm, SAS at 0.60%, PAA at 30ppm achieved a 3-log reduction in all strains after 5 minutes (p<0.05). No significant differences in corrosion rates were determined between SS304 and SS316 (p<0.05). Temperature and sanitizer type influenced corrosion rates significantly (23|73 / Chlorine (4.02E-05 |1.68E-04), PAA (1.11E-04| 8.03E-04), SAS (1.03E-04 |8.15E-04), Water (4.03E-04 | 2.12E-03)cm/year) (p<0.05). AL3003-3004 showed greater corrosion than SS, with the highest rates determined for the 0.6%-SAS treatment (4.52E-02cm/year).
Significance: All sanitizers at the highest concentrations achieved a 3-log reduction with a 5-min contact time per EPA requirements. These findings don't reflect current grower practices. Soil-plant health will be impacted by aluminum pipe corrosion.

P3-142 Investigation of *Salmonella* Internalization Intricacies in *Arabidopsis thaliana*

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❖ ***Developing Scientist Entrant***

Introduction: Concerns of *Salmonella* contamination on fresh produce, are heightened in hydroponic farming systems, where there is a higher chance of *Salmonella* internalization into plants, but the data of such internalization has been inconsistent.
Purpose: This study aimed to develop a more reliable and quantifiable plant internalization test assay to address these inconsistencies, and further investigate the mechanism of *Salmonella* internalization.
Methods: The *Arabidopsis thaliana* plants were cultivated vertically for seven days, and *Salmonella* (8 log CFU/plant) was directly applied to the roots using filter paper slides. Two days post inoculation, ten plants were enumerated with peptone water as one sample. Next, the internalization of *Salmonella* virulence mutant strains (*S.* ΔfliC and *S.* ΔprgH) was compared in such test assay. Additionally, plants were cultivated at different temperatures (18 and 25 degrees) and *Salmonella* internalization was investigated with different plant stomata number mutant lines. One data point was calculated from means and standard deviations of six replicates. Student's t-test was applied for statistical analysis.
Results: *Arabidopsis thaliana* exhibited a high level of *Salmonella* internalization at 6.49 ± 0.22 log CFU/plant with this assay. In the subsequent study

examining *Arabidopsis* immunity and *Salmonella* virulence, unlike the *S.* wild strain and *S.* ΔprgH, *S.* ΔfliC did not trigger an immunity response upon leaf contamination. Conversely, mutant strains of different *Salmonella* virulence including *S.* ΔfliC and *S.* ΔprgH internalized into *Arabidopsis* via roots at similar levels to the wild strain ($p > 0.05$; 5.27 ± 0.70 log CFU/plant, 5.91 ± 0.46 log CFU/plant and 5.94 ± 0.69 log CFU/plant, respectively). Subsequent trials with diverse temperature conditions and *Arabidopsis* mutant lines indicated that such internalization could be related to both the cultivation temperature and plant stomata numbers.

Significance: This study focused on the intricate internalization of *Salmonella* in the model plant *Arabidopsis*, laying the groundwork for future research and the development of innovative strategies to mitigate foodborne pathogen internalization.

P3-143 Deciphering the Dynamics of Attachment and Internalization of mScarlet-I Labeled (Chromosomally Integrated) *S. enterica* Oranienburg and Shiga Toxin-Producing *E. coli* O157:H7 (STEC) in Kale

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Introduction: The internalization of foodborne pathogens such as *E. coli* O157:H7 and *Salmonella enterica* in leafy greens presents health risks. These enteric pathogens can contaminate fresh produce, posing a substantial health threat. The rising popularity of kale for its nutritional benefits highlights the importance of understanding its interactions with these pathogens.

Purpose*:* This study aimed to clarify the varying interaction dynamics of different kale genotypes with STEC and *S. enterica*.

Methods*:* Both pathogens were chromosomally tagged with mScarelet I and the integration, fitness, and stability of the labels were confirmed through PCR, TEM, and physiological assays. We assessed the survival, attachment, and internalization of chromosomally tagged STEC and *S.* Oranienburg in three kale varieties (Starbor F1, Vates Blue Scotch Curled (VBS), and KX-1 F1) at pathogen concentrations of 10^6 and 10^8 CFU/ml- by different methods including syringe infiltration and dipping the leaf surface for 5 s (Jacob et al., 2017). Following independent inoculations, we quantified microbial population survival at 10 dpi using microbial plating, stereozoom fluorescent microscopy, and SEM. The experiments were repeated 2 times, 3 replicate per variety (3 replicates/plant) and statistically analyzed using ANOVA and Tukey's test ($\alpha < 0.05$).

Results*: S.* Oranienburg exhibited significantly higher attachment to KX-1 and VBS varieties ($p<0.0001$), ranging from 5.97±0.366 to 3.4±0.39 CFU/cm². STEC also showed higher attachment to these varieties ($p<0.0001$), ranging from 5.65±0.15 to 3.9±0.69 CFU/cm², with Starbor F1 having significantly less ($p<0.0001$) for both pathogens. STEC's survival was consistent across all kale varieties, while *S.* Oranienburg displayed a significant difference ($p<0.0064$) between Starbor F1 and VBS. Significant internalization differences ($p<0.0001$) for both pathogens were confirmed by SEM. Internalization of *S.* Oranienburg was significant ($p=0.03$) between Starbor F1 and KX-1, and STEC showed a significance difference ($p<0.0001$) between KX-1 and VBS, with significance differences in VBS and Starbor F1 ($p=0.0004$).

Significance: Exploring these interactions provides crucial insights for devising strategies to prevent future outbreaks.

P3-144 Factors Influencing Antibiotic-resistant Bacteria in Urban Agriculture Environments

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❖ ***Developing Scientist Entrant***

Introduction: Urban agriculture supports food security and provides benefits to local communities. While organic amendments are commonly used to restore depleted soil health, implications for dissemination of antibiotic-resistant bacteria remain unclear.

Purpose: To determine how soil amendments, among other factors, associate with concentrations of antibiotic-resistant bacteria across urban farms and community gardens.

Methods: We conducted a microbiological field survey of leafy vegetables grown organically in greater Washington, D.C. (n=7 sites). Samples of leaf tissue (n=92), amended soil (n=92), and bulk soil (n=39) were collected and analyzed for concentrations of total bacteria, ampicillin-resistant (Amp) and tetracycline-resistant (Tet) bacteria, and coliforms. Non-parametric statistical tests were applied to determine differences in respective bacteria concentrations based on site-specific factors. Isolates representing distinct colony morphologies were further screened to characterize multidrug resistance in the urban food systems.

Results*:* Across-site differences in bacterial contents of samples reflected general land history and crop management, while within-site variation was attributed to amendment types, plant growth stage and, to a lesser extent, vegetable type or variety. Concentrations of total bacteria in amended soils were significantly greater than those in bulk soils at all farm sites ($p<0.05$ for each site). The 'priming effect' associated with reduced fractions of Tet bacteria, as well as case-specific reductions in Amp bacteria and coliforms. Nevertheless, we observed high frequencies of multidrug resistance among the Amp (67.3%; n=110) and Tet (77.2%; n=101) isolates. As soil properties such as pH strongly correlated with Tet bacteria in amended soils ($rho=0.529$; $p<0.001$), tailored crop management holds potential to control antibiotic-resistant bacteria and emerging multidrug resistant strains.

Significance: Our findings demonstrate that organic amendments promote soil bacteria and, in turn, may suppress food safety risks. Future research on these microbial interactions will aim to develop bio-based strategies to improve safe and sustainable food production in urban environments.

P3-145 Development of a Rapid Enteric Pathogen Indicator for Leafy Greens

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Introduction: *Salmonella* and STEC contamination is a major concern in fresh leafy green products. Testing for pathogens and indicator organisms such as coliforms and *E. coli* can take up to 24 hours or more to return results. Methods with faster turnaround times are sorely needed for produce, which has short shelf life and very tight supply chain timelines.

Purpose: To develop a molecular method that detects indicators of enteric pathogen contamination and requires less than six hours of enrichment.

Methods: Samples of 19 different leafy green and other fresh produce matrices were artificially inoculated (7 test portions, each of 375 g per matrix; 133 portions in total) with < 30 CFU of *Salmonella* and/or STEC and enriched in pre-warmed buffered peptone water containing 0.5% polysorbate-20. Samples were incubated at 42°C for 5.5 hours, and then the entirety of each enrichment was concentrated using the Pathotrak™ concentration system. Concentrated samples were tested using the Enteric Pathogen Risk Indicator (EPRI) real-time PCR kit (Gold Standard Diagnostics) in parallel with being tested for both *Salmonella* and STEC by real-time PCR.

Results: For all 19 matrices tested, all inoculated replicates produced a positive detection using EPRI real-time PCR following 5.5 hours of enrichment and sample concentration. The presence of *Salmonella* and/or STEC was confirmed by pathogen-specific PCR in each sample. Thus, every matrix passed the FDA matrix extension protocol requiring detection in 7/7 inoculated samples. There was no observed inhibition from the produce or concentration procedures in the PCR reaction.

Significance: These data demonstrate the feasibility of a rapid protocol to assess the potential presence of enteric pathogens in fresh produce matrices. If the EPRI assay indicates that enteric pathogens may be present, this data demonstrates that further testing of the concentrated sample for *Salmonella* and/or STEC is also possible.

P3-146 Surveillance and AMR Detection of Microbial Communities in Farms Across the Mid-Atlantic

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Introduction: Microbial surveillance in farm soils can advance our understanding of the presence of pathogens. Information gathered upon sampling, along with detection of bacterial pathogens and antimicrobial resistance (AMR) genes can inform pre-harvest food safety guidance.

Purpose: This study presents data correlating bacteria and AMR genes detected in soils, with observational and climatic information.

Methods: Soil samples (n=174) were collected from active fields during July-October 2023 from six farms across the Mid-Atlantic. Soil was taken at a 7cm depth around legumes, nightshades, brassica vegetables, and melons. Soil samples were massaged gently with Universal Pre-enrichment broth or BPW+beef extract. Samples were analyzed for *E. coli* and coliforms. Following TET and RV incubations, *Salmonella* was enumerated on XLT-4. Soil was incubated in buffered *Listeria* enrichment broth and plated onto modified oxford agar. AMR genes (*blaCTX-m*, *tetA*, *gyrase A*, and *mcr-1*) were detected by qPCR. Statistical analysis to determine correlations between environmental factors and bacterial levels was performed using JMP software and Microsoft Excel.

Results: Soil samples (13.8%) were positive for *E. coli*, with one third of those positives coming from one particular farm. *L. monocytogenes* was detected in all soil samples. Soil samples (31%) were positive for *Salmonella*, and 74% of these positive samples originated from three farms. Rain, proximity to woods and proximity to manure piles are factors that increased bacterial presence; with a moderate positive correlation between rainfall prior to sampling and the log (CFU/g) of Salmonella detected (r = 0.339). Of the *Salmonella* isolates tested, all were positive for *mcr-1* and *blaCTX-m* genes, 75% were positive for *tetA*, and 67% were positive for *gyrase A*. Indicating resistance to colistin, beta-lactamases, tetracycline, and fluoroquinolones.

Significance: Surveillance of farm soil samples combined with metadata can be used to enhance our understanding of pathogen presence, AMR, and connections to climate and on-farm conditions.

P3-147 Fungi and STEC Analysis of Maine Wild Blueberries in 2022 and 2023

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Introduction: Wild blueberry production in Maine is a vital part of the local economy, providing a significant source of income for farmers and contributing to agricultural diversification in the region. However, ensuring the food safety of these wild fruits is a growing concern, especially in the context of the Food Safety Modernization Act (FSMA), legislation that seeks to improve food safety standards.

Purpose: The aim of this study is to perform a comprehensive microbiological analysis of wild blueberries produced in Maine, with special focus on fungi and Shiga toxin-producing *Escherichia coli* (STEC) detection.

Methods: Soil, fruit, bins, conveyer belts and harvesters were sampled weekly on four (4) different farms during harvest seasons in 2022 and 2023. Fungi were enumerated culturally and STEC was detected using a selective enrichment/qPCR approach. Fungi counts analysis in samples over weeks was performed using linear regression, modeling log-transformed variable as a function of time and source. Positive STEC results were analyzed using chi-square to measure the effect of location and sample type.

Results: Analysis indicated a statistically significant (p<0.05) increase of fungi count as the season progressed. Soil and post-harvest fruits samples (introduced in 2023) reaching 4.13 and 4.06 log CFU/g, respectively, demonstrated significantly higher counts than (2.95 log CFU/g). There wasn't significant difference in mean fungal counts (within sample type) between 2022 and 2023. Proportional positive STEC results for type vary significantly between years but not for farm. Harvesters represented the highest proportion in 2022 and post-harvest fruits in 2023, indicating the pathogen presence is related to the type of sample.

Significance: A detailed understanding of blueberry microbiology is essential to develop management strategies and agricultural practices that ensure compliance with the guidelines established by FSMA. Preventing foodborne illness outbreaks, while controlling the quantity of fungi is important to preserve quality and attractiveness, minimizing spoilage effects.

P3-148 Ecological Distribution of *Staphylococcus* in Integrated Farms within Washington D.C.–Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Mixed crop-livestock farms (MCLFs) are integrated food production systems where both livestock and crops are grown near each other to promote environmental sustainability through recycling of waste material. However, livestock of MCLFs are reservoirs of many zoonotic pathogens including *Staphylococcus*, and their predominance can alter the farm microbial eco-system and cross-contaminate food products.

Purpose: To determine the prevalence of various staphylococcal species in the MCLF ecosystem within the DC-Maryland metropolitan area and their antibiotic-resistance patterns.

Methods: A total of 3038 environmental and pre-harvest produce samples and 836 post-harvest produce samples were collected from eight MCLFs and two retail supermarkets, as well as 36 skin swabs from farmworkers and non-farmworkers were collected. *Staphylococcus* was detected by culturing and confirmed with PCR, and further identification of staphylococcal species was confirmed by a multiplex PCR. Antibiotic-resistance profiles were determined by an antibiogram assay using clinically relevant antibiotics.

Results: The overall prevalence of *Staphylococcus* was 12.18% in environment/pre-harvest samples and 7.54% in post-harvest samples. The most identified species was *S. epidermidis* (19.86%), though most isolates remained unknown (73.90%). Approximately 83.33% of the skin swabs were positive for *Staphylococcus*, with *S. xylosus* being the predominant species (16.7%). Among the three sample types, a paired t-test was used to find that the prevalence of unknown *Staphylococci* was significantly higher (p<0.05). However, *S. epidermidis* was significantly more prevalent compared to the other species that were identified. The highest percentage of resistance was to aminoglycosides and macrolides, with 24.11% of tested samples being multidrug resistant. *S. epidermidis* had the most resistance compared with the other species.

Significance: Antibiotic-resistant *Staphylococcus* are present in MCLF environments, and proper steps are needed to control the transmission of this pathogen among livestock, crops, and humans.

P3-149 Efficacy of *Lactobacillus rhamnosus* and Its Metabolites to Mitigate the Survival of Foodborne Pathogens in Hydroponic Nutrient Solution

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❖ ***Developing Scientist Entrant***

Introduction: Hydroponic nutrient solution (HNS) has been established to be an ideal conduit for pathogen contamination and proliferation.

Objective: To determine the efficacy of *Lactobacillus rhamnosus* and its metabolites to inhibit the survival of foodborne pathogens in HNS when compared to conventional treatments.

Method: Hoagland's HNS that were prepared according to the manufacturer's instructions were inoculated with *Salmonella* Typhimurium, *Escherichia coli* 0157:H7, and *Listeria innocua* at 10^5 CFU/ml cell concentration. These nutrient solutions were subjected to treatment with various concentrations of *L. rhamnosus* live cells, a cell-free extract (CFE) of *L. rhamnosus* metabolites, and peroxyacetic acid (PAA) at 22±1°C for up to 96 h, and survivors (both patho-

gen(s) and *L. rhamnosus*) were enumerated on respective selective media at regular intervals. The physico-chemical properties of treatment solutions, such as pH, electrical conductivity, salinity, total dissolved solids, and % lactic acid content, were determined using standard procedures. All the experiments were conducted in triplicates. Survival data was log transformed and analyzed using two-way ANOVA on JMP pro 16 with a significance level of 0.05.

Results: Both *S.* Typhimurium and *E. coli* O157: H7, when in combination with *L. rhamnosus,* remained stable in HNS over a 96-h period, while *L. innocua* showed a 3-log reduction. CFE treatment of HNS showed a significant reduction in *Salmonella* and *E. coli* O157: H7 (both undetectable after 96 h; LOD: <1 log CFU/ml). Interestingly, *L. innocua* levels remained stable after CFE treatment. PAA treatments at 12 mg/L notably reduced *Salmonella* and *L. innocua* growth, but not *E. coli* O157:H7.

Significance: These findings showed promise to utilize *Lactobacillus* metabolites as an alternative sustainable antimicrobial intervention to reduce the risk of foodborne pathogens in HNS. Further studies are warranted to understand the effect on plant growth and the composition of extracted metabolites.

P3-150 Optimizing Ultraviolet Treatment for Hydroponics to Improve Crop Safety and Yield

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Introduction: Preliminary studies have shown that ultraviolet treatment is able to reduce microbial contamination in the nutrient water of hydroponic systems. However, it is not known how these ultraviolet treatments may impact nutrient water chemistry or crop growth and yield.

Purpose: The objective of this study is to examine the impact of an ultraviolet light treatment on romaine lettuce growth parameters and nutrient levels in the treated hydroponic water.

Methods: **Commercially available** DWC (Deep Water Culture) hydroponic systems were used to grow romaine lettuce (*Latticua lettuceia* var. Sparx) in a nutrient solution containing Hydro-Gro Leafy (8.87%) and calcium nitrate (6.96%); the electrical conductivity was maintained between 1.6 and 1.8 mS/cm. The nutrient solution was treated with a UV-C device (MiniPure MIN-1; 500ml capacity) emitting peak irradiance at 254nm at flow rates of 0,3 and 6 L/min. Three samples were collected before and after each treatment and the experiment was repeated three times. Twice weekly during the six-week growth period, parameters including plant height, leaf width, SPAD value, chlorophyll fluorescence, and leaf length were measured. Following harvest, parameters including fresh weight and dry weight were measured. Statistical analysis will include one-way ANOVA followed by Tukey's honestly significant difference test at p of less than or equal to 0.05 to determine differences in means of growth parameters between treatment groups.

Results: Low and high UV doses resulted in 1.17 and 1.36 log reductions of *Escherichia coli* in hydroponic nutrient water. Preliminary findings for the effect of UV light on the concentration of nutrients (NPK) yielded no significant difference in the nutrient level. The study is underway for assessing the effect on the lettuce growth parameters.

Significance: UV light technology at optimized dosage levels has the potential to improve the safety of hydroponic systems with minimal effect on the plant growth and nutrient water.

P3-151 Evaluation of the in Vitro Potential of *Bacillus Subtillis* and v*elezensis* against *Salmonella* Typhimurium as Candidate Biocontrol Agents

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Introduction: Biocontrol agents, specifically Plant Growth Promoting Rhizobacteria (PGPR) like Bacillus spp. and Pseudomonas spp., have been extensively researched due to their dual advantages in promoting plant growth and controlling phytopathogens. Despite this, there exists a notable gap in scientific literature regarding their impact on human pathogens and their interactions with plants. The rising instances of outbreaks and recalls linked to Controlled Environment Agriculture (CEA)-grown produce underscore the critical need to address microbial safety in fresh produce.

Purpose: The objective of this study was to investigate the *in vitro* biocontrol potential of two known plant growth-promoting strains, *Bacillus subtilis* 3A25 and *Bacillus velezensis* 3A37, against *Salmonella* Typhimurium under controlled conditions (35°C).

Methods: Employing a cell density procedure and a modified disk diffusion method, the study aimed to assess the *in vitro* activity of the selected Bacillus strains against a representative strain of multidrug-resistant *Salmonella* Typhimurium, *Salmonella enterica* subsp. enterica serovar Typhimurium strain G11013 (ATCC BAA-190™). This was done in six replications. Counts were log transformed and subjected to one-way ANOVA.

Results: The assay revealed that at p<0.05 significance level, both Bacillus strains exhibited a significant reduction in S. Typhimurium counts over a 48-hour incubation period compared to the control group without PGPR. *B. velezensis* strain demonstrated a noteworthy 2 log CFU/L reduction, while B. subtilis showed a 1 log reduction, with statistical significance observed at 24 hours (p<0.05). However, the disk diffusion method did not show clear inhibition zones for either Bacillus strain against S. Typhimurium. Therefore, while the antagonism study suggested limited direct effectiveness against S. Typhimurium, the observed reduction in pathogen cell density implies potential indirect effects.

Significance: These findings, coupled with subsequent in vitro and in vivo studies, aim to assist growers in determining whether specific PGPR strains could function as a co-management strategy for both phytopathogens and foodborne pathogens, offering a biocontrol option and providing valuable insights for best industry practices.

P3-152 The Utilization of Bacteriophage as Biocontrol Agent to Proactively Prevent Escherichia coli Contamination in Microgreen Cultivations

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❖ ***Developing Scientist Entrant***

Introduction: Microgreens, primarily cultivated in greenhouses, are highly susceptible to foodborne pathogens and surface bacterial colonization.

Purpose*:* Assess the T7 phage's antimicrobial potential against *Escherichia coli* contamination and examine *Escherichia coli*'s death rate after phage treatment on microgreens' surface.

Methods: Three 3cm x 3cm pre-trimmed microgreen leaves were washed for one minute with sterile DI water to remove soil particles and sanitized with 70% ethanol to eliminate background flora. After a rapid burn-dry to remove the residual alcohol and the air-dry process, 15 μl of 8 log CFU/ml of *E. coli* BL21 was spot-inoculated onto a single spot on the leaves, followed by a 30-minute incubation, respectively. Post-incubation, treatment groups were inoculated with T7 phage at a 1:5 MOI ratio. Treatment groups were inoculated with the pathogen and 75 μl of 9 log PFU/ml of T7 phage, while the control groups were inoculated with the pathogen and sterile saline. Both sets of samples underwent incubation for 0, 2, and 4 hours at 25°C and 37°C with added sterile DI water. Subsequently, both samples underwent bead beating with silica beads, followed by serial dilution for bacterial enumeration.

Results*:* Recovered *E. coli* BL21 counts remained at 6 log CFU/ml in all control samples. A significant (p<0.05) reduction in *E. coli* BL21 counts was observed in microgreens treated with T7 phage across all 24 replicates. Quantitative analysis revealed a consistent 3-log reduction of *E. coli* BL21 in the treatment group compared to the controls during the 2-hour and 4-hour incubations at 25°C and 37°C, respectively, with the reduction rate persisting up to 4 hours. Additionally, consistent 1.5 to 2-log reductions of *E. coli* BL21 were shown in all the treatment groups from 0-hour to 4-hour intervals.

Significance: This novel approach assures mitigation of pre-harvest microbial contamination, specifically against *E. coli* contamination in microgreens. The findings advance proactive bio-control strategies, enhancing food safety in microgreen production.

Poster

P3-153 Unraveling the Potential of Plant Growth-Promoting Rhizobacteria (PGPR) for Enhanced Lettuce Growth and Food Safety

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Introduction: Understanding the intricate interactions between plant growth promoting rhizobacteria (PGPR) and plants is essential for developing strategies to promote crop growth and resilience and safeguard agricultural produce safety.
Purpose: Investigate PGPR's potential for enhancing lettuce growth and food safety when plants are under drought-induced abiotic stress.
Methods: Rhizobacteria (n=35) were isolated from roots of bok choy (*Brassica rapa* subsp. *chinensis*) grown at a University of Maryland Farm. Bacterial isolates were taxonomically classified following full-length 16S rRNA gene sequencing. Romaine lettuce seedlings (cvs. 'Parris Island Cos', 'Rouge d'Hiver') were inoculated 3- and 6-days post-germination with single rhizobacterial strain suspensions of 7 log CFU/plant. Romaine lettuce plants (n=4) were screened for growth promotion by fresh and dry biomass analysis at 4 weeks. Isolates facilitating positive lettuce growth effects were further screened for their potential to restrict *Salmonella* under plant water-restriction or regular watering, with or without PGPR colonization (n=6). Leaf populations of *Salmonella* Enteritidis were enumerated by direct plate counting on Tryptic Soy Agar 24 h after leaf inoculation with 5 log CFU.
Results: Twenty-one out of 35 isolates significantly increased Romaine lettuce growth compared to untreated plants (p<0.05). Isolate BC37 (*Pseudomonas frederiksbergensis*) increased the fresh weight of 'Parris Island Cos' after 21 days of inoculation (p<0.001) compared to no PGPR control plants, suggesting its potential as a robust PGPR. *P. frederiksbergensis* was further evaluated for modulatory effects on lettuce-*Salmonella* Enteritidis association with two Romaine lettuce cultivars. Root inoculation with *Pseudomonas* BC37 restricted *Salmonella* population inoculated on leaves of 'Parris Island Cos' (p<0.001) but not 'Rouge d'Hiver', compared to controls. The reduction in *Salmonella* populations on *Pseudomonas*-treated plants experiencing water stress was slightly greater compared to uninoculated plants, with differences of 1 log or less observed in both plant varieties.
Significance: Our study explores the potential for harnessing microbial interactions for sustainable and safe crop production in the face of environmental challenges.

P3-154 Bridging the Gap by Listening to the Needs: A Case Study With Military Veteran Farmers

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❖ ***Developing Scientist Entrant***

Introduction: Military veteran farmers constitute a niche group who encounter unique barriers in obtaining food safety education. Previous research has shown that food safety training can achieve better outcomes when materials are tailored to the intended audience.
Purpose: This two-part study was conducted to identify the on-farm food safety practices and resource needs of U.S. military veterans who are farmers.
Methods: In study 1, an online survey was administered in March 2023, and was divided into demographics, farming background, food safety knowledge, attitudes, practices, perceived behavioral control, and food safety education needs of participants. The survey was distributed nationwide using a list of military veteran farmers through the Farmer Veteran Coalition. A paired sample t-test was used to compare mean scores for reliable food safety sources. Study 2 consisted of in-person interviews conducted on a multi-state level in 2022–2023 at regional and national agricultural conferences.
Results: In study 1, a total of 668 military veteran farmers attempted the survey, of which 550 (82%) were suitable for analysis. A majority (98%) grew or planned to grow food crops. Only 51% of participants previously had received food safety training, among whom only 30% had Good Agricultural Practices training and even fewer (10%) had Produce Safety Alliance training. The reported format preferences for on-farm food safety education were email (n=343, 62%), in-person workshops (n=337, 61%) and online videos (n=308, 56%). Participants rated University Extension as the most reliable source for food safety information, surpassing government sources, farmers' market managers, third-party auditors, and farmer peers (p <0.001). Study 2 encompassed a series of interviews (n=27), in which military veteran farmers identified lack of time as a significant barrier to accessing on-farm food safety education. Virtual reality (VR) was identified as a "potential tool" but only if it can better accommodate farmers with disabilities.
Significance: The findings will guide the development of food safety educational materials for military veteran farmers.

P3-155 Evaluation of Knowledge, Attitudes and Practices Towards Farm Food Safety in Singapore Farmers

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Introduction: With the growing demand for local produce and increasing emphasis on food safety from farm to fork, this study examined the Knowledge, Attitudes, and Practices (KAP) related to farm food safety within Singapore's farming community.
Purpose: Establish a baseline for KAP regarding farm food safety in local farmers, aiming to identify key intervention areas and enable the evaluation of future intervention measures.
Method: A structured questionnaire, based on the Singapore Standards for each farm type, was prepared. The questionnaire was administered to 84 farmers across 48 farms, involving a manager and a supervisor from each farm for well-rounded perspective. Questions were categorized into three groups: farm management, equipment and materials handling, and produce handling. Quantitative analysis was utilized to explore variations in KAP scores across different farm types and demographic factors and, statistical analyses highlighted significant disparities and correlations between various KAP scores and demographic factors.
Results: Layer farms outperformed others in all evaluated categories, setting a benchmark for farm practices. In agriculture, a significant majority (93%, 55/59) of smaller farms failed to implement practices reducing heavy metal soil contamination, with a direct correlation (r=1) between this non-adoption and a lack of understanding, notably among less educated farmers. Over 50% of aquaculture farmers did not comply with documentation requirements, and a moderate correlation (r=0.45) was observed between unfamiliarity with veterinary drug usage and non-compliance. In layer farms, more than half of the farmers (63%, 5/8) reported non-conformance with bird quarantine practices despite default compliance, indicating a substantial knowledge-practice discrepancy.

Significance: This study presented useful behavioral perspectives and insights to inform policy and targeted measures which would overall contribute to further enhancing farm food safety practices in Singapore and elsewhere.

P3-156 A Systematic Review on Pre-harvest Interventions Used to Control *Salmonella* in Poultry in the United States

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Introduction: *Salmonella* contamination in poultry represents a significant public health concern. Pre-harvest interventions can play a significant role in reducing *Salmonella* loads in poultry before entering the processing establishment. However, a comprehensive understanding of pre-harvest interventions used to control *Salmonella* in poultry in the United States (US) does not currently exist.
Purpose: This systematic review seeks to identify and synthesize existing literature on pre-harvest interventions and their effectiveness in the control of *Salmonella* in poultry in US.
Methods: Utilizing the PRISMA framework, a systematic search of peer-reviewed literature was conducted across major scientific databases including Web of Science, Scopus, PubMed, Agricola, CAB abstracts and Food Science and Technology Abstracts. Experimental studies assessing pre-harvest inter-

ventions to control *Salmonella* in poultry in U.S poultry farms published from 1995 to date were identified and included in the review if they reported prevalence (qualitatively or quantitatively) and levels of *Salmonella*. Data extraction employed a double-entry method, and the extracted data were compiled in an Excel spreadsheet. Descriptive statistics were used to summarize key study parameters, including study design, study location, poultry type, *Salmonella* serotypes, type of intervention, and effectiveness of intervention.

Results: A total of 12,403 studies were identified, and 234 were included in the review. Evaluated interventions included feed/water additives (31.5%), competitive exclusion culture (14.8%), vaccination (6.4%), prebiotics/probiotics (5.1%), bacteriophages (2.1%) and antibiotics (0.8%) among others. Most studies focused on chicken (86.8%) and the most common *Salmonella* serotypes investigated were Typhimurium (40.4%), Enteritidis (31.5%), and Heidelberg (6.4%). Overall, the effectiveness of evaluated interventions varied among studies due to differences in study design, sample sizes and duration of interventions.

Significance: This review improves the understanding of the effectiveness of pre-harvest interventions in controlling *Salmonella* in poultry. Findings will help inform food safety policies and practices around poultry, ultimately protecting public health.

P3-157 Prevention of Virus Internalization in Hydroponic Leafy Greens by Heat Treatment

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Introduction: The number of farmers growing hydroponic vegetables is gradually increasing due to advantages of rapid plant growth, water-saving, and easy handling. In spite of advantages of hydroponic culture, there is a probability of viruses to be transferred from the hydroponic system into edible tissues of plant if water is contaminated.

Purpose: The aim of this study was to examine whether murine norovirus (MNV) can be internalized in leafy vegetables through contaminated hydroponic solution and heat treatment can inactivate MNV in hydroponic solution.

Methods: In the first study, lettuce was grown for 5 days in hydroponic solution contaminated with 10^{3-4} plaque forming units/ml of MNV. Viral RNA was extracted daily from lettuce or hydroponic solution (each 10 samples) and detected using RT-PCR. In the second study, MNV-contaminated hydroponic solution was heated at 55–95 ℃ for 3 min and used in lettuce culture for 5 days. MNV was quantified by RT-qPCR (the limit of detection = 1×10^2 genome copies) on a daily basis. A result was demonstrated in triplicate from three independent trials. The differences were evaluated with Duncan's multiple range test and the values were assessed significantly different at the $P < 0.05$.

Results: When MNV-contaminated hydroponic solution was used, MNV was detected daily in plant tissues (an average of 7/10) and hydroponic solution (10/10). After heat treatment at 55–65 ℃, there was no reduction effect compared to the control. However, a reduction of 1.22 log was shown at 75 ℃ and MNV titers were below the limit of detection above 85 ℃ in hydroponic solution. In addition, no MNV was recovered in lettuce samples (0/10) grown with heat-treated hydroponic solution.

Significance: This result showed that waterborne viruses could remain in hydroponic solution for days and eventually be internalized into plant if water source was contaminated with viruses. Additionally, heat treatment of hydroponic solution can reduce virus internalization in leafy greens.

P3-158 Surveillance of *E. coli* O157:H7 and *Salmonella Typhimurium* in Hydroponic Systems at Different Electric Conductivity Levels in Nutrient Solution

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Introduction: To meet the projected global population of 9.75 billion by 2050, agricultural innovations, particularly Controlled Environment Agriculture (CEA) hydroponic techniques, have gained popularity, especially in cultivating leafy greens. However, the increased consumption of fresh produce has raised concerns about foodborne illnesses linked to pathogens in hydroponic greenhouses.

Purpose: This research focuses on understanding the survival of *E. coli* O157:H7 and *Salmonella* Typhimurium in hydroponically grown lettuce under different electrical conductivity levels in the nutrient solution.

Methods: Lettuce plants grown in Nutrient Film Technique (NFT) system under conditions to mimic greenhouse conditions in California. Different electrical conductivity levels in the nutrient solution (1.2 $mS \cdot cm^{-1}$, 1.7 $mS \cdot cm^{-1}$, and 2.2 $mS \cdot cm^{-1}$) were inoculated with *E. coli* O157:H7 or *Salmonella Typhimurium* after 2 weeks of seedling germination. Water, roots, and leaves were sampled at 0, 3, 6, 12, and 24 hours, 2, 7, 14, 21, and 28 days. Three replications and statistical analyses with R was conducted.

Results: Results showed no significant difference in pathogen inoculation under three electrical conductivity (EC) levels ($p<0.05$). Both pathogens behaved similarly in the nutrient solution, with no significant difference observed within the initial 12 hours. By day 7, *Salmonella* Typhimurium was undetectable ($p<0.05$), while *E. coli* O157:H7 exhibited consistent growth ($p<0.05$). Leaf samples were devoid of microorganisms, while in root samples, *Salmonella Typhimurium* reached 3 log within 24 hours, no countable but persistence after day 7 ($p<0.05$). *E. coli* O157:H7 persisted in root samples, reaching 3 log in all treatments, with a ~2 log increase from day 7 onwards ($p<0.05$).

Significance: The study shows foodborne pathogens surviving in the NFT system's nutrient solution for up to 28 days. Varying EC levels do not control pathogen survival. Pathogen migration to roots highlights the need for nutrient solution management to mitigate contamination risks in hydroponic systems.

P3-159 *Listeria monocytogenes* Contamination of Blossoms Yields Contaminated Strawberries

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Introduction: *Listeria monocytogenes* causes listeriosis, a severe, foodborne illness with high hospitalization and mortality rates. Outbreaks of listeriosis have been associated with fresh produce, including stone fruits. One possible source of contamination is blossom exposure. Strawberries can serve as a model and may be vulnerable to contamination as overhead irrigation is used for frost protection.

Purpose: Understanding how produce contamination occurs can aid prevention.

Methods: Blossoms were inoculated with an erythromycin resistant *L. monocytogenes* strain, derived from an outbreak isolate. An exponential phase culture was adjusted to one of three inoculum levels. A 10µL droplet, of *L. monocytogenes* or sterile saline, was spotted on blossoms and the resulting berries were harvested for detection. Enrichment was performed in brain heart infusion (BHI) broth with erythromycin and each was plated for enumeration. After 48 h at 37°C, counts were determined, and *L. monocytogenes* was detected using Rapid'L.mono agar.

Results: A single trial was performed for the higher inoculums, while the low inoculum was tested in two trials. In low inoculum (3-7 CFU) trials, only one blossom of 36 in the first trial and one of 25 in the second yielded *L. monocytogenes* at harvest. Conversely, a moderate inoculation (~700 CFU) resulted in four of six blossoms yielding *L. monocytogenes*, two without enrichment, while 24 of 26 blossoms inoculated with the high inoculum (~10^5 CFU) yielded *L. monocytogenes*. Of the *L. monocytogenes* positive berries, only four required enrichment to detect *L. monocytogenes* and eight had ≥3000 CFU/g. No control blossoms (n=80) yielded *L. monocytogenes*, including 39 control blossoms on plants receiving *L. monocytogenes* on neighboring blossoms.

Significance: While further studies are needed, these data indicated that *L. monocytogenes*-contaminated water contacting blossoms can result in contaminated fruit at harvest but reducing *L. monocytogenes* in the water could reduce the risk. Additionally, control blossom data suggested that direct contact with irrigation water is necessary for contamination.

Poster

P3-160 Assessment of *Escherichia coli* (*E. coli*) and *Salmonella enterica* on Fresh Vegetables Grown on Crop-Only Farms vs. Integrated Crop-Livestock Farms in Siem Reap, Cambodia

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Introduction: Cambodian farms often integrate crop and livestock production. Many foodborne pathogens regularly inhabit the intestinal tracts of livestock. As such, vegetables grown in close proximity to livestock production may be more frequently contaminated with foodborne pathogens.

Purpose: To assess the prevalence of *E. coli* and *Salmonella enterica* (*Salmonella*) on fresh vegetables, specifically cucumbers and lettuce, food contact surfaces (FCS), and non-food contact surfaces (NFCS) from farms with crop-only or integrated crop-livestock production systems in the Siem Reap Province, Cambodia.

Methods*:* A total of 216 samples were collected from 36 crop-only farms and 36 integrated crop-livestock farms in Siem Reap between April and November 2022. *E.coli* and *Salmonella enterica* were cultured from each sample using traditional methods. Putative isolates were confirmed to the species level by RT-PCR.

Results: No significant difference (p=0.2589, estimate [95% CI]) was observed in the overall prevalence of *E. coli* between samples originating from crop-only (31%) vs. integrated crop-livestock (40%) farms. Lettuce had the highest prevalence of *E. coli* in samples from both crop-only farms (44.4%) and integrated crop-livestock farms (61.1%) compared to all other sample types (Cucumber from crop-only farms: 11.1%; cucumber from integrated crop-livestock farms: 11.1%). The contamination rate of *E. coli* on FCS and NFCS (FCS: 13.8%, NFCS: 7.4% from crop-only farms and FCS: 50%, NFCS: 16.6% from integrated crop-livestock farms). *Salmonella enterica* was recovered from only two samples from crop-only farms and four samples from integrated crop-livestock farms. This low prevalence of *Salmonella enterica* prevented robust comparisons across sample types.

Significance: This study did not identify any differences in *E. coli* or *Salmonella enterica* contamination rates on vegetables (lettuce and cucumber) produced on crop-only farms vs. integrated crop-livestock farms. Further research may focus on spatial, temporal, and behavioral factors and how they may factor into contamination rates in vegetables produced on the different types of farms.

P3-161 Impact of the Application of Commercial Probiotic Cultures on the Survival of *Listeria monocytogenes* in Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* contamination in fresh produce has been a significant public health concern. The ability of *L. monocytogenes* colonizing on different food contact and non-food contact surfaces and surviving and growing at low temperatures have made the control of *L. monocytogenes* contamination in fresh produce challenging.

Purpose: The objective of this study was to evaluate the efficacy of using commercial probiotic cultures (SafePro®) to control the survival of *L. monocytogenes* in fresh produce.

Methods: Fresh cut Romaine lettuce and baby spinach obtained from local grocery store were confirmed to be negative for *L. monocytogenes* before use, they were then inoculated with a cocktail of rifampin-resistant *L. monocytogenes* at ca. 4.0 Log CFU/g. After inoculation, commercial SafePro® culture was applied at the level of 7.0 log CFU/g. The surviving *L. monocytogenes* as well as the counts of viable SafePro® culture were determined by plating on modified oxford agar and De Man–Rogosa–Sharpe agar. The visual properties of the fresh produce were examined as well during storage.

Results: *L. monocytogenes* grew in fresh produce during refrigerated storage. When inoculated with only *L. monocytogenes*, the *L. monocytogenes* in cut Ramine lettuce and spinach increased from ~4 Log CFU/g to 5.2 Log CFU/g after 7 days of storage. By Day 16 (end of storage), levels of *L. monocytogenes* in both produces ranged from 5.3-5.8 Log CFU/g. When co-inoculated with SafePro®, the presence of probiotics slowed down the proliferation of *L. monocytogenes.* By the end of storage, the *L. monocytogenes* levels were significant lower (*P- value* < 0.05) in produce co-inoculated with LM and SafePro® (0.4-0.8 Log CFU/g) than the ones inoculated only with LM.

Significance: Commercial SafePro® cultures demonstrate it promising potential in inhibiting the growth of *L. monocytogenes*. It also benefits the overall quality of fresh produce. Additional research is still needed to further investigate the feasibility and strategies to implement protective cultures at processing plants.

P3-162 Effect of Extended Refrigerated Storage on *Escherichia coli* O157:H7 Survival and Virulence on Romaine Lettuce and Packaging Film

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❖ ***Developing Scientist Entrant***

Introduction: Despite continuous advancements in inactivation methods, *Escherichia coli* O157:H7 contamination of fresh produce, including lettuce, continues to pose a significant concern. This issue underscores the susceptibility to contamination by pathogens and highlights the limitations of current post-harvest pathogen inactivation and sanitation practices. Simultaneously, it emphasizes the critical need for more effective approaches to ensure food safety.

Purpose: This study aimed to evaluate the survival and virulence of *E. coli* O157:H7 post-harvest on romaine lettuce leaves and packaging films at 4°C over a 15-day period.

Method: The survival of *E. coli* on romaine lettuce leaves and packaging films at 4°C over different periods was measured by plate counting and PMAx PCR. The expression of virulence and stress response genes was quantified using qPCR. The infectivity efficacy of the *E. coli* cells was measured by an attachment assay using the HT-29 cell line.

Results: Log reductions occurred on successive days from 0 to12, with no detectable cells on day 15 on non-selective agar plates. However, PMAx qPCR revealed stressed viable cells on day 15. Gene expression studies showed a consistent upregulation of virulence genes (*stx1a, stx2a, fliC*, and *eaeA*) over 15 days. The universal stress gene *uspB* exhibited variable upregulation and increased expression in lettuce but was downregulated in the packaging film. Furthermore, our results indicate that cold exposure of lettuce leaves enhances *E. coli* attachment to mammalian cells (e.g., 2.4×10^5 on day 0 vs 3.8×10^5 on day 6).

Significance: These findings suggest that *E. coli* survival exhibits a time-dependent pattern during extended refrigerated storage. These data could be useful for post-harvest risk assessment of *E. coli* in romaine lettuce.

P3-163 Survival and Growth of *Salmonella enteritidis* and *Escherichia coli* O157:H7 in Alfalfa and Mung Bean Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: Consumption of sprouts has grown globally, principally driven by their perceived nutritional and health benefits. Over 60 foodborne illness outbreaks worldwide have been linked to sprouts. Home-scale sprout production has become increasingly popular, yet. There is a lack of science-based evidence on the survival of enteric pathogens in home-like settings.

Purpose: This study sought to examine the prevalence of the most implicated food-borne pathogens, *Salmonella enteritidis,* and *E. coli* O157:H7, during the lifecycle of alfalfa and mung bean sprouts exposed to 5 LOG CFU/ml in home-like settings and conditions (25°C, 70 ± 5% RH, and double daily rinsing).

Methods: Disinfected seeds of alfalfa or mung beans were inoculated with *Salmonella enteritidis* or *Escherichia coli* O157:H7, and the seed/sprout and lid surface were sampled over seven days (sprouts cycle) for pathogen enumeration. *Ten replicates were made for each microorganism, type of sprout, and sample type during the seven days.* Three-way ANOVA with a significance level of (p<0.05) was used for statistical analysis.

Results: There was statistical significance (p<0.05) that showed interaction between the three variables (type of sprout, sample type, and microorganism). Both microorganism and sprout types had an initial decline of 2 to 4 LOG/CFU, followed by an increase in pathogen growth between 2 and 4 LOG CFU/ml between days 2 and 4 (depending on the variable combination). At the end of the seven days, both microorganisms survived between 4.8 and 6.2 LOG/CFU in the sprout and lid surfaces.

Significance: The pathogens evaluated in the two sprouts remained in the seeds and lid surface in harmful concentrations for humans until day seven at a harmful concentration for human health.

P3-164 Survival of *Listeria monocytogenes* and *Salmonella* on Frozen Raspberry and Blueberry

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❖ ***Developing Scientist Entrant***

Introduction: Frozen produce has been associated with *Listeria monocytogenes* and *Salmonella* outbreaks and recalls.

Purpose: This study sought to determine *L. monocytogenes* and *Salmonella* survival on raspberries and blueberries under frozen storage.

Methods: Ten-gram samples of fresh raspberry and blueberry were spot inoculated with 10 µL (3-5 drops) of a five-strain *L. monocytogenes* or *Salmonella* cocktail at approximately 6.0 log CFU/fruit (n=10). Prior to freezing, berry samples were air-dried in a biosafety cabinet for 30 min. Samples were frozen at -20°C and enumerated in duplicate at the following time-points: 0, 1, 2, 3, 5 and 30 d. At each time-point, frozen samples were thawed in a water bath for 8±2 min at 25°C and mixed with 20 mL of buffered peptone water. Samples were stomached for 90 s at low-speed serial diluted in 0.1% peptone water and plated on selective (modified oxford agar and xylose lysine deoxycholate) and non-selective media (tryptic soy agar) + rifampicin (80ppm) + 0.1% sodium pyruvate. Data were analyzed in R-Studio (V.4.3.1) using the Tukey's HSD test to assess significant differences (P≤0.05).

Results: *L. monocytogenes* and *Salmonella* decreased on frozen berry samples over the 30-d storage. No significant pathogen reductions were observed between each time-point for raspberry and blueberry samples. By 30 d, *L. monocytogenes* concentrations reduced by 0.5±0.2 and 0.5±0.4 log CFU/fruit on raspberry and blueberry samples, respectively. *Salmonella* concentrations reduced by 0.5±0.2 and 0.5±0.4 log CFU/fruit on raspberry and blueberry samples, respectively, by 30 d.

Significance: Even in the absence of growth and with a decrease in recoverable concentrations over time, *L. monocytogenes* and *Salmonella* demonstrated survival for a minimum of 30 d in frozen storage of berries. Findings emphasize the importance of enforcing rigorous control measures to avert or reduce the presence of pathogens prior to or during the freezing of berries.

P3-165 Survival and Recovery of *Escherichia coli* O157:H7 and *Salmonella enterica* Serovars in Microgreens Irrigated with Contaminated Water

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Introduction: Microgreen consumption has increased tremendously around the globe as consumers are becoming aware of the health benefits including the bioactive compounds they contain. However, there are recalls associated with these commodities, leading to increased safety concerns.

Purpose: Irrigation water is a resource that has been identified as a point of entry for several foodborne pathogens in leafy greens. This study aims to determine the transfer of enteric pathogens to microgreens irrigated with contaminated water.

Methods: Municipal water inoculated with a 3-log CFU/ml cocktail of two strains of *Salmonella enterica* or *E. coli* O157:H7 was used to irrigate seeds of daikon, red cabbage, broccoli, and mustard, grown on soil beds on days 0 and 3. On days 7 and 14, microgreen and soil samples were harvested, pummeled in TSB (5X volume), and subjected to MPN and spot plating on XLT4 and SMAC. Seeds and soil samples were confirmed to be free from these pathogens prior to use in the study.

Results: Both *Salmonella* and *E. coli* showed a decrease in the prevalence over 14 days across all four microgreens. On day 7, ~1.5 log CFU/g of both the pathogens was recovered from the four microgreens whereas 4-4.3 log CFU/g was recovered on average from the soil beds. On day 14, ~1 log CFU/g of both the pathogens was recovered from the microgreens and 3.7-3.9 log CFU/g was recovered on average from the soil beds.

Significance: Controlled environment agriculture is helping to manage climate change related uncertainties, where resources and environmental conditions can be monitored. If contaminated water is used to irrigate the microgreens, the pathogens can be internalized, causing serious consequences to human health.

P3-166 Survival of *Escherichia coli* O157:H7, Microbiome Shift and Persiser Cell Development on Romaine Lettuce under Source and Forward Processing Conditions

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Introduction: Multiple *Escherichia coli* O157:H7 (EcO157) outbreaks associated with romaine lettuce have been reported in recent years, which might be linked to the recurring contamination of persistent EcO157 strains. Our previous study indicated delayed processing and air pressure fluctuation during transportation of forward processed products.

Purpose: To assess the survival of EcO157 and microbial dynamics on romaine lettuce under simulated conditions for source processing (SP, local) and forward processing (FP, remote).

Methods: Fresh harvested romaine lettuce from California was shipped overnight to ARS laboratory. The whole head romaine lettuce was inoculated with EcO157 outbreak strain 2705C or reference strain EDL933. Inoculated leaf samples were tested on day 0 (IN, initial sampling conducted immediate after vacuum cooling), day 1 (SP simulation), and day 8 (FP, simulation with air pressure fluctuation; AT, alternative test without air pressure change) post

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inoculation. EcO157, aerobic bacteria (AC), yeast and mold (YM) counts were enumerated, and microbial DNAs extracted for microbiome analyses targeting bacterial 16S rRNA and fungal ITS genes. Percentage of persister EcO157 cells under SP and FP conditions were analyzed based on ciprofloxacin and ampicillin treatment.

Results: The populations of both EcO157 strains were higher on lettuce under SP conditions than those on FP and AT samples (p<0.05). No significant differences between SP and FP samples for AC and YM populations. In addition to inoculated EcO157, *Pantoea* and *Pseudomonas* were the dominant bacteria, and the major fungal genera were *Filobasidium*, *Sporobolomyces*, and *Vishniacozyma*. Alpha diversity of FP and AT bacterial communities increased after an additional one-week of storage (p<0.05), while the diversity of fungal communities was not significantly changed. The rate of persister EcO157 cells from FP lettuce was significantly increased compared with SP samples (p<0.05).

Significance: Data derived from this study provide insights for the development of preventive controls on EcO157 contamination during romaine lettuce production.

P3-167 *Escherichia coli* Transfer Onto and Internalization into Strawberries Dropped on Plastic Mulch

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❖ Developing Scientist Entrant

Introduction: The US FDA Produce Safety Rule prohibits the distribution of dropped-covered produce due to the risk of contamination from the ground and the potential for impact damage to increase the internalization of pathogens.

Purpose: Evaluate *Escherichia coli* transfer and internalization potential to strawberries dropped onto plastic mulch from various heights.

Methods: Unwashed strawberries (n=192), randomly selected and weighed, were dropped over both new and used plastic mulch using PVC pipes of varying heights (15.24, 30.48, 60.96, 121.96 cm). Mulch was spot-inoculated with gfp-tagged *E. coli* (ca. 8 log CFU) and dried for 1h. Bacterial transfer (BT) to fruit surfaces was assessed via plate count. To measure bacterial internalization (BI), fruit surfaces were sterilized, prior to homogenization and plate counts followed by enrichment to identify the presence of *E. coli.* Three independent experiments with four replicates were conducted (n=16); BT and BI percentages were calculated. One-way ANOVA found significant differences (p>0.05) among scenarios; linear regressions examined correlations between bacterial BT/BI rates and fruit weight.

Results: *E. coli* survived significantly better during drying on new plastic mulch (7.6 ± 0.25 log CFU/mulch) than used mulch (6.9 ± 0.58 log CFU/mulch) (p<0.05). CFU transfer to strawberry surfaces was significantly elevated (p<0.05) from new mulch (2.62 ± 1.6% to 8.75 ± 3.77%) compared to used mulch (0.018 ± 0.01% to 16.46 ± 15.38). Though internalized bacteria were minimal (less than 0.7 Log CFU/strawberry), the presence of *E. coli* was detected in strawberries dropped onto new mulch dropped from 15.24, 30.48 and 121.96 cm, and onto used mulch when dropped from 15.21 and 121.92 cm. No correlation was observed between with BT or BI and weight.

Significance: Higher bacterial survival following inoculation and transfer to strawberries was seen from new plastic mulch, emphasizing the importance of not harvesting dropped strawberries.

P3-168 Survival of *Salmonella* on Packinghouse Conveyor Belts

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❖ Developing Scientist Entrant

Introduction: Fresh produce foodborne outbreaks have been linked to cross-contamination within packinghouses. Food contact surfaces in packinghouses, such as conveyor belts, pose a food safety risk due to the persistence of microbes in difficult to clean and sanitize areas.

Purpose: This study evaluated *Salmonella* survival on conveyor belts used in commercial packinghouses.

Methods: Polyurethane conveyor belts sourced from a commercial packinghouse were constructed into 5x5cm coupons. Coupons were surface sterilized using 50ppm bleach and spot inoculated with a five strain 80ppm rifampicin-resistant Salmonella cocktail at approximately 8 log CFU/ml. Inoculated coupons were air dried for 30 min and stored in a growth chamber maintaining 55% RH and 22°C. Coupons were enumerated in triplicate at ten time-points including 0, 0.04, 0.25, 1, 2, 3, 4, 7, 14, and 42d, and plated in duplicate on Tryptic Soy Agar with 80ppm rifampicin. Descriptive statistics and significant differences were evaluated using Tukey's HSD Test (p≤0.05) in R studio (V4.2.3).

Results: Salmonella survived 42d on packinghouse belt coupons. Significant differences were observed between mean log CFU/coupon and the time-points 0, 0.04, 0.25, and 1d (p<0.05), with an initial total reduction from 0 to 1d of 1.8±0.7 log CFU/coupon. Following the initial reduction, no significant differences were observed between mean log CFU/coupon and time-points 1, 2, 3, and 4d (p>0.05). Significant reductions in Salmonella populations were not observed between time-points (p>0.05). By 42d, Salmonella concentrations on coupons had reduced by a total of 4.1±0.0 log CFU/coupon.

Significance: Considering Salmonella survived 42d, it is important that conveyor belts be frequently cleaned and sanitized to reduce potential cross-contamination of Salmonella with fresh produce.

P3-169 Survival of *Listeria monocytogenes* on D' Anjou Pears Co-Inoculated with *Bacillus thuringiensis*, *Aerobasidium pullulans*, and *Penicillium expansum* during 70 Days of Cold Storage

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❖ Developing Scientist Entrant

Introduction: Pears may be stored under long-term storage conditions for up to nine months before consumption. However, the impact of common industry storage practices on pear commensal organisms and foodborne pathogens is still unknown.

Purpose: To determine the survival of *Listeria monocytogenes* (LM) on pears co-inoculated with *Bacillus thuringiensis*, *Aerobasidium pullulans*, and *Penicillium expansum* during 70 days of cold storage following industry practices.

Methods: D'Anjou pears (n = 9 per treatment and storage time point) were assigned to twelve treatment combinations based on co-inoculation type [LM-only control, co-inocula *B. thuringiensis* (BT), *A. pullulans* (AP) or *P. expansum* (PE)], wound status (intact or wounded), and storage conditions [bulk or wrapped with tissue paper impregnated with copper carbonate (1.3%) and ethoxyquin (0.1%)]. A 6.25 cm^2 pear surface was spot inoculated with 100 µL of LM cocktail, air dried for one hour, and then co-inoculated with 100 µL of the respective co-inoculum. LM and co-inocula populations were determined at 0, 7, 14, 21, 28, 42, 56, and 70 days of cold storage by plating on Chromagar *Listeria* (LM), Mannitol Egg Yolk Polymixin (BT) or Dichloran Rose Bengal Chloramphenicol agars (AP and PE). When LM was below the limit of detection (2.67 log CFU/pear), the most probable number (MPN) method was used.

Results: After 70 days of storage, LM, BT, and PE populations significantly decreased on pears wrapped with tissue paper (p<0.001). LM population was reduced by ~2.5 log CFU/pear on wrapped pears inoculated with LM-only or co-inoculated with AP or PE (p<0.001). In bulk pears, LM populations were similar, regardless of wound status and co-inoculation type, except when co-inoculated with PE. In this case, only in wounded pears, the population of PE significantly increased, whereas LM decreased to levels below the LOD.

Significance: Tissue paper containing copper and ethoxyquin could help further reduce LM and PE decay concerns in pears.

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P3-170 Microbial Surveillance of Fruits and Vegetables in a South American Country

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Introduction: Fruits and vegetables can become contaminated with foodborne pathogens during production, harvesting and packing. Knowing the pathogen contamination levels in different produce products is important to develop risk-based inspection decisions for a developing country.

Purpose: To identify product-specific pathogen contamination levels in key fruit and vegetable commodities to support ongoing surveillance and regulatory initiatives in a South American country.

Methods: At least 25-g sample of each produce commodity was collected monthly from different points of sale since 2019 until 2023 (n= 600). Samples were homogenized with 225-ml of BPW for 2-min at 230 RPM. Serial dilutions were made for generic *E. coli* enumeration, in addition, pathogen detection and isolation were conducted in selective media. Both *Salmonella* and *Escherichia coli* data were analyzed using R (Version 4.2.2) software to quantify *E. coli* and evaluate the change in *Salmonella* prevalence for different type of samples on an annual basis.

Results: *E. coli* quantification data showed that in 2019 blackberries, garlic, strawberries, and lettuce had contamination loads of 1.17, 2.08, 1.84 and 1.38 log CFU/10g, respectively. Lower loads for blackberries and garlic were observed in 2022, with 0.70, 0.69 log CFU/10g, while strawberries and lettuce had 0.57, and 0.65 log CFU/10g, respectively in 2023. As for cilantro the higher contamination was observed in 2022 with 2.25 log CFU/10g and lower contamination in 2023 with 0.93 log CFU/10g. Onions in 2023 had a higher contamination with 1.12 log CFU/10g that 2022. In general, *Salmonella* prevalence in blackberries lettuce, cilantro, strawberries, and onions was very low throughout all years evaluated.

Significance: Data describing the prevalence and loads of pathogens in produce at the point of consumption is necessary for designing adequate surveillance systems, and potential regulatory schemes. Risk-based inspection systems can benefit from surveillance programs that identify contamination trends throughout different timeframes, processor types, and commercialization venues.

P3-171 Prevalence of Pathogenic Microorganisms in Agricultural Production Environments in Queretaro, Mexico: a Correlation of Good Agricultural Practices Compliance, Technological Level, and Seasonality

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Introduction: Implementing Good Agricultural Practices (GAP) effectively minimizes biological, chemical, and physical hazards during fresh produce growing. The environment, water, and infrastructure at production facilities are also important factors for food safety.

Purpose: To evaluate the association of GAP compliance, technological level, and prevalence of foodborne pathogens at four agricultural production sites (APS) in Queretaro, Mexico.

Methods: The study was conducted in an open field farm (APS1), a greenhouse (APS2), and two hydroponic greenhouses (APS3 and APS4), having low, medium, medium, and high technological levels, respectively. Each APS was visited twice during autumn, winter, spring, and summer; temperature (ambient, soil, water), humidity (ambient, soil), pH (soil, water), and GAP compliance were registered. Soil (n=11), agricultural water (AgWA, n=30), iceberg lettuce (n=24), Roma tomato (n=18), strawberry (n=24), and cherry tomato (n=24) samples were collected and analyzed for *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* detection. In addition, generic *E. coli* was quantified in AgWA (n=49), non-AgWA (n=8), and fresh produce (n=90).

Results: From the evaluated parameters, only temperature and pH of AgWA varied among the APS. GAP compliance at APS1, APS2, APS3, and APS4 were 20%, 45%, 42%, and 93%, respectively. *E. coli* O157:H7 and *L. monocytogenes* were absent, whereas *S. enterica* was detected in three AgWA samples from APS1, APS2, and APS3, and in one APS2 soil sample (4/131, 3%). Generic *E. coli* was present in AgWA of APS1 (43%), APS2 (45%), and APS3 (50%). Microbial Water Quality Profile based on FSMA Produce Safety Rule revealed that the four APS met the standard during production and only AgWA of APS4 was suitable for harvest and post-harvest operations. A positive correlation was observed between GAP compliance and technological level (r=0.97).

Significance: Adequate implementation of GAP was related to APS´s level of technology and did not change during seasons. Strategies to prevent AgWA contamination during harvest and postharvest must be reinforced to guarantee produce safety.

P3-172 *Salmonella* and Levels of Indicator Microorganisms in Fresh Berries Sold at Retail Markets

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Purpose: Assess the frequency of *Salmonella* and indicator microorganism levels in fresh berries sold at retail markets in Guadalajara, Mexico.

Introduction: Mexico is an important producer and exporter of berries. Berries can be sold in wholesale and retail markets, including fresh markets, supermarkets, and street sales. During its marketing, berries can be kept at temperatures that favor the permanence of microorganisms and are exposed to microbial contamination, affecting their quality and safety.

Method: A total of 212 berries samples were collected from retail markets in Guadalajara, Mexico between April 2022 to January 2023. Fifty-three samples of each type of berries: blueberries, blackberries, raspberries, and strawberries were analyzed. Each sample (50g of whole fruits) was rinsed in 450 mL of Buffered Peptone Water and homogenized by ultrasound bath (300W, 40 kHz, 1 min) for *Salmonella* detection by BAM-FDA culture method and surface levels of aerobic plate count, *Enterobacteriaceae*, coliforms, *Escherichia coli*, yeasts and molds. The percentage of positive samples for *Salmonella* and indicators was reported. Indicator counts were converted to log CFU/mL of rinse liquid, and mean values ± three standard deviations (SD) were calculated.

Results: *Salmonella* was detected in one (0.89%) strawberry sample. Mean levels of indicators (Log CFU/mL of rinse liquid) were 4.2 ± 4.5 for aerobic plate counts, 0.8 ± 2.9 for Enterobacteriaceae, 0.3 ± 2.5 for coliforms, -0.3 ± 0.6 for *Escherichia coli*, yeasts and molds 3.9 ± 4.4 and 2.2 ± 4.2 respectively. Low levels of *Escherichia coli* were recovered only in 9 samples corresponding to blackberries (n=3), strawberries (n=1) and raspberries (n=5).

Significance: Berries commercialized in retail markets can be vehicle of *Salmonella* and variable levels of indicators. The variability among the indicator counts and pathogen in the berries suggest diverse handling conditions at stages of the productive chain.

P3-173 Assessing Salmonella Transfer from Wastewater to Hydroponic Lettuce in a Pilot-Scale Bioponic System

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❖ ***Developing Scientist Entrant***

Introduction: Bioponic systems have demonstrated efficient nutrient recovery from wastewater for hydroponic irrigation, however, the impact of pathogens poses significant food safety risks.

Purpose: This study evaluated the fate of *Salmonella* influx in a pilot-scale wastewater treatment system utilizing poultry processing wastewater for lettuce production.

Methods: The treatment system comprised of bioreactors inoculated with consortia of algae and nitrifying bacteria, clarifiers, membrane filters, UV disinfection, and hydroponic grow beds. Bioreactors were dosed with *Salmonella* at two concentrations: 3 and 5 log CFU/ml, daily. Water and lettuce

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samples were analyzed across three stages: pre-inoculation (days 0-10), inoculation (days 11-20), and post-inoculation (days 21-30). Water samples were serially diluted and plated for *Salmonella* enumeration. Lettuce samples were homogenized, suspended in buffered peptone water, enriched, plated, and presumptive colonies were confirmed.

Results: At the lower concentration (3 log CFU/ml), pre-inoculation samples showed no detection of *Salmonella*. During inoculation, direct plating yielded no *Salmonella* counts in water samples, but enrichment revealed its presence. UV disinfection eliminated *Salmonella* from water samples, with no detection in lettuce. There was no detection of *Salmonella* in both water and lettuce samples at the end of the recovery phase (day 30). When challenged with higher *Salmonella* concentration (5 log CFU/ml), pre-inoculation samples showed no detection of *Salmonella*. By day 15 of inoculation, *Salmonella* counts in reactors, clarifiers, and filters were reduced to approximately 3 log CFU/ml. Although no *Salmonella* was detected via direct plating in UV effluents and holding tanks, enrichment methods indicated potential pathogen persistence or reactivation. By the end of the recovery phase, the system had restored to baseline pathogen levels, with no detection of *Salmonella* in UV effluents, grow beds, and lettuce samples.

Significance: This study confirms the effectiveness of the treatment system in managing varying *Salmonella* loads, enhancing food safety, and sustainable agriculture practices.

P3-174 Prevalence of *Escherichia coli* and *Salmonella enterica* in Fresh Vegetable and Environmental Samples from Farm to Market in the Province of Battambang, Cambodia

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Introduction: In Cambodia, the lack of hygienic practices and insufficient infrastructure throughout the vegetable value-chain increases the risk of bacterial contamination of raw vegetables, which may increase the incidence of foodborne disease in the country.

Purpose: To determine the prevalence of *E. coli* and *Salmonella* on lettuce, tomato, cucumber, and environmental samples (food contact [FC] and non-food contact [NFC] surfaces) at three key points in the Cambodian vegetable value chain, namely farms, distribution centers [DC], and markets.

Methods: A total of 720 (480 vegetable and 240 environmental) samples were collected from farms, DC vendors, and market vendors in Battambang Province during the dry and rainy seasons. Presence of *E. coli* and *Salmonella* was assessed with traditional culture methods and confirmed with RT-PCR.

Results: For *Salmonella,* the highest prevalence was observed on FC surfaces (estimate [95% CI]: 10 [4, 21] % and NFC 7 [3, 16] %), regardless of season and location in the value chain. Vegetable samples were rarely positive, with the highest prevalence being observed in lettuce samples (5/90). For *E. coli,* regardless of season and location, prevalence was highest in lettuce (31 [20, 43] %) relative to all other sample types.

Significance: In this study, the overall prevalence of *Salmonella* along the vegetable value chain was considered low, whereas the prevalence of *E. coli* was considered substantial. Findings showed no evidence for differences between value-chain locations or season on *E. coli* or *Salmonella* contamination of samples. Findings further highlighted lettuce as a common reservoir for *E. Coli* contamination, and FC and NFC surfaces as common reservoirs for *Salmonella*.

P3-175 Characterization of *Escherichia coli* and *Listeria monocytogenes* isolated from Wastewater Systems in a Fresh Produce Processing Facility and the Downstream Food Safety Impact

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Introduction: Commercial fresh produce processing facilities follow stringent hygienic practices that involve washing, rinsing, cleaning, and disinfecting to eliminate potential foodborne pathogens. Quaternary ammonium compounds, found in the disinfectants used in the food industry, have traditionally been perceived as chemicals with the potential to provide selective pressure on bacterial strains with acquired resistance to other antimicrobials.

Purpose: The study aimed to assess the contribution of food safety-control practices to the downstream quality of wastewater from a food processing facility and its potential environmental and public health impact.

Methods: 153 Water samples were collected over four seasons from different sections of a commercial fresh produce processing facility in South Africa. Standard microbiological quality methods were used to determine hygiene indicator bacteria (coliforms/*E.coli*) counts. The presence of *Listeria monocytogenes* and potential pathogenic *E. coli* were analyzed through selective enrichment, plating onto chromogenic media, and identity confirmation using MALDI-ToF analysis. Phenotypic antimicrobial resistance profiles were confirmed through double-disk diffusion and all isolates (n= 14) were subjected to WGS (Illumina MiSeq) and analyzed using Galaxy.

Results: The input chlorinated water had coliform and *E. coli* counts <1,8 log MPN/100ml. Drain water post-processing had coliform and *E. coli* counts ranging between 0,3 - >6 log MPN/100ml and 0 – 2,84 log MPN/100ml, respectively. After enrichment, *L. monocytogenes* (n= 5) was isolated from the drain that received water from the low and high care sections for the duration of the study. All the isolated *E. coli* had multidrug resistance antibiograms and genes conferring resistance to third-generation cephalosporins were present. Two *E. coli* ST10 strains were identified, however, none of the strains harbored STEC-associated virulence genes.

Significance: This study contributes to expanding water quality and antimicrobial resistance gene dissemination knowledge in fresh produce processing facilities as a basis for developing and implementing food safety strategies.

P3-176 Persistence and Cross-Contamination of *Listeria monocytogenes* on Avocados in the Food Environment

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✦ ***Undergraduate Student Award Entrant***

Introduction: Listeria monocytogenes outbreaks linked to packaged fresh produce (2018-2019 US recalls of avocados) need to be prevented. Contaminated surfaces can potentially cross-contaminate fresh produce and vice versa in produce packing houses. Factors that enhance its ability to survive, adhere, and transfer to fresh produce need to be investigated.

Purpose: The objectives of this research were to (1) Determine the survival of L. monocytogenes serotype 4b on avocado skins over 2 weeks at room temperature (25□, 44% relative humidity (RH)) and at refrigeration (4□, 76% RH); and (2) Determine its transfer from a contaminated stainless-steel surface (SS) to avocados at room temperature.

Methods: Twelve avocados were surface sanitized before inoculation with 100 µL overnight culture mixed with autoclaved soil (1:10), air-dried, aseptically transferred to sterile containers, and stored at 25□ or 4□ for 14 days. Bacteria were recovered (including un-inoculated control avocados) using phosphate-buffered saline, and ten-fold serial dilutions were surface spread plated on tryptic soy agar and incubated at 37□ for 24 to 48 h before enumeration. Transfer studies involved taking 3 cleaned, autoclaved SS coupons or avocados and inoculating 2 with the bacterial-soil mix and drying for 30 min at room temperature (25□, 44% RH) within sterile Petri dishes. Transfer experiments were done by placing un-inoculated or inoculated individual avocados on inoculated or uninoculated coupons, respectively. Data from triplicate experiments were assayed in duplicate and statistically analyzed.

Results: L. monocytogenes (~8.5 log CFU) mixed with soil could persist on avocados under both temperature and humidity conditions for 2 weeks. Cross-contamination studies showed that 6.79±1.07 log CFU of L. monocytogenes could be recovered from contaminated SS that could transfer 5.80±2.04 log CFU to avocados, while 5.88±1.25 log CFU could be transferred to SS from 7.34±0.98 log CFU recovered from contaminated avocados.

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Significance: This data is useful to understand survival and transmission for risk-assessment analysis and implementation of appropriate control practices for food processing.

P3-177 Transfer of *Salmonella enterica* and *Enterococcus faecium* from Food-Contact Surfaces to Stone Fruits

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Introduction: Contaminated food-contact surfaces are a potential route for spreading microorganisms to stone fruit during postharvest handling.
Purpose: The objective of this study was to investigate the factors that affect the transfer of bacteria from food-contact surfaces to stone fruits.
Methods: Coupons (1 × 1 cm) of polyurethane (PU) or polyvinyl chloride (PVC) were inoculated with either a five-strain rifampin-resistant *Salmonella* cocktail or *Enterococcus faecium* NRRL B-2354 (EF) at 5 or 7 log CFU/cm². Inoculated coupons (n = 8–11) were attached to a texture analyzer, and uniform contact conditions (5 N, 5 s) were used to explore the impact of inoculation level, bacterial species, donor surface, recipient surface structure (peach or nectarine), and the presence of dry wax or peach juice on the transfer rate of bacteria. Whole fruits were transferred to 20 mL of 0.1% peptone, rubbed for 2 min, and then the diluent was plated onto trypticase soy agar supplemented with rifampin at 50 µg/mL. Whole fruits were enriched when populations were anticipated to fall below the limit of detection (1.9 log CFU/fruit). Rates of transfer (%) are the ratio of populations on the recipient fruits and donor surfaces.
Results: At an inoculum of 5 log CFU/cm², *Salmonella* and EF were recovered from the fruit by enrichment but not by plating. At 7 log CFU/cm², rates of transfer (0.04–0.26%) were not significantly ($p > 0.05$) influenced by either bacterial species or donor surface. The rates of transfer of EF from contaminated PU to peaches or nectarines (0.05–0.07%) were not significantly different. The rates of transfer of EF increased significantly ($p < 0.05$) in the presence of wax (17.46%) or peach juice (1.26%) on the PU surface compared with the control (0.08%).
Significance: The presence of residues on food-contact surfaces facilitates the movement of microorganisms during dry handling of fresh stone fruits.

P3-178 Changes in Injured *Salmonella* and *Listeria monocytogenes* Populations during Storage of Cold Plasma and Organic Acid Treated Tomatoes

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Introduction: The behavior of injured *Salmonella* and *Listeria monocytogenes* on tomato surfaces following treatment with cold plasma and organic acid sanitizer during storage is needed to understand efficacy of the antimicrobial treatments.
Purpose: To determine the efficacy of cold plasma and sanitizer treatments on injury and survival of *Salmonella* and *Listeria* on tomato.
Methods: Tomato surfaces were inoculated with *Salmonella* and *Listeria monocytogenes* at 7 logs by spotting and total submersion of produce for 3 min to achieve averaged 5.7 and 5.3 logs/g of each pathogen, respectively. Pathogen-inoculated tomatoes were treated with cold plasma, organic acid, and a combination of the two treatments for 0, 120 s and 180s. Tomato samples were then homogenized in neutralizing buffer and 100 µL aliquots of treatment samples were plated on selective and non-selective agar (37°C for 24h), to assess bacteria populations. Data recorded were converted to log CFU/g, and data were analyzed with analysis of variance (ANOVA) to assess significance of treatment effects (SAS Institute, Cary, NC).
Results: The surviving *Salmonella* populations on tomatoes treated with cold plasma for 120s and 180s averaged 2.2 and 1.4 logs, respectively and populations were significantly ($p<0.05$) lower (1.6 and 1.1 logs) on tomatoes treated with organic acids. When tomatoes were treated with cold plasma for 120s and 180s, injured *Salmonella* populations averaged 43.6% and 49.2%, respectively; and were 29.6% and 32.7% on organic acid treated tomatoes. Combination treatments for 120s and 180s reduced the surviving populations (both pathogens) to < 0.6 logs and had significant ($p<0.05$) reductions of injured populations to <10%, which died off on tomato stored at 4°C for 24h and at 20°C for 36h.
Significance: The results suggest that cold plasma and organic acid treatment combination and immediate tomato storage at refrigeration temperature will inhibit recovery of injured pathogens on tomatoes designated for fresh-cut salad.

P3-179 Dry-Heat Treatment in Reducing *Salmonella* and *E. coli* O157:H7 Contamination on Alfalfa Seeds

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Introduction: The Produce Safety Rule requires seeds for sprouting be treated by either sprout growers or seed suppliers to reduce pathogens. Dry-heat treatment can be an option for seed suppliers as it is scalable and avoids the need for a post-treatment drying step.
Purpose: Evaluate the efficacy of dry heat in reducing *Salmonella* and *E. coli* O157:H7 on alfalfa seeds, as affected by temperature (60, 70, 80°C), relative humidity (20-80%), time (6, 16, 24 h) and treatment scale (10 g, 1 kg). Determine the impact on germination, sprout yield and pathogen re-growth during sprouting.
Methods: Ten g of inoculated seeds were subjected to dry-heat treatment in a humidity-controlled chamber. Treated seeds were analyzed for *Salmonella* or *E. coli* O157:H7 by plate count and culture enrichment. One hundred seeds were germinated in a petri dish and percent germination was recorded for 5 days. Sprout yields were determined after 7 days. For large-scale treatment, 1 kg seeds containing 10% of inoculated seeds were treated. Pathogen re-growth was examined by sprouting 200 g of treated seeds in glass jars for 5 days and pathogen levels were analyzed daily.
Results: Greater log kills were achieved when treatment was conducted at higher temperatures, higher relative humidities (RH), or for longer time. Optimal conditions that reduced *E. coli* O157:H7 to below detection (< -0.3 log CFU/g) or *Salmonella* by > 3 logs while maintaining germination and sprout yield at > 90% were identified (70°C/40%RH for 16 or 24h). A similar log kill was achieved whether 10 g or 1 kg of seeds were treated. Large-scale treatment under the optimal condition reduced *Salmonella* to below detection (< -0.3 log CFU/g), but pathogen re-growth (by > 6 logs) was observed during sprouting.
Significance: Dry-heat treatment could reduce *Salmonella* and *E. coli* O157:H7 on seeds to below detection, but pathogen re-growth could occur during sprouting.

P3-180 Impact of Contact Time on Transfer of Generic *Escherichia coli* to Fresh Cucumber, Jalapeño, and Tomato

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❖ ***Developing Scientist Entrant***

Introduction: The food safety risk of produce attached to the plant and in contact with the ground is unknown, as produce that has dropped to the ground cannot be harvested under the Food Safety Modernization Act Produce Safety Rule.
Purpose: The objective of this study was to assess surface transfer of generic *Escherichia coli* (g*EC)* from plastic mulch to cucumber, jalapeño, and tomato at different contact times.
Methods: Plastic mulch coupons were inoculated with rifampicin-resistant, green fluorescent protein-tagged g*EC* at 5 log CFU/cm². Coupons were dried for 24h (55% RH, 23°C) before placement in the field. Field-grown cucumber, jalapeño, and tomato that were in contact with the inoculated dried coupons were randomly assigned time-points (0, 1, 3, 5, and 7d) in a research plot (n=15 each). At assigned time-points, each commodity and corresponding mulch

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coupon were enumerated for surface transfer of g*EC*, and enriched if counts were below the limit of detection using the FDA BAM. Descriptive statistics and significant differences in g*EC* by commodity and contact time ($p \leq 0.05$) were determined by Tukey's HSD test in RStudio (V4.2.3).

Results: Significant differences were observed between day 1 and day 3 cucumber log percent transfer with no trend in contact time ($p<0.05$, range: <1.30, 5.44 log CFU/cucumber). No significant differences were observed between contact time and log percent transfer of jalapeño or tomato ($p>0.05$, range: <1.30, 3.52 log CFU/ jalapeño and <1.30, 3.53 log CFU/tomato). Generic *E. coli* log percent transfer of cucumber samples was significantly higher ($p<0.05$) or equivalent to those of jalapeño and tomato samples for all contact times.

Significance: If produce was in contact with contaminated areas (simulated by g*EC* inoculated plastic mulch coupons), transfer occurred more frequently than not. While contamination was minimally influenced by contact time, contamination varied by commodity (cucumber ≥ jalapeño ≥ tomato).

P3-181 Effect of Drop Height on Transfer of Generic *Escherichia coli* to Fresh Cucumber, Jalapeño, and Tomato

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❖ *Developing Scientist Entrant*

Introduction: Dropped-covered fresh produce is prohibited from harvest due to potential microbial contamination per the Food and Drug Administration's Food Safety Modernization Act Produce Safety Rule.

Purpose: This study investigated the surface transfer of generic *Escherichia coli* (g*EC)* at different drop heights from plastic mulch to cucumber, jalapeño, and tomato and biodegradable mulch to tomato.

Methods: Rifampicin-resistant, green fluorescent protein-tagged g*EC* was spot-inoculated on plastic mulch coupons. Before use, coupons were dried for 24h (55% RH, 23°C). Five drop heights (0, 1, 2, 4, and 6ft) were assigned to cucumber, jalapeño, and tomato samples and dropped through height-modified PVC pipes onto dried inoculated plastic mulch at approximately 5 log CFU/cm^2 (n=15 each). Fifteen additional tomato samples were dropped on biodegradable mulch coupons following the same protocol. Transfer was evaluated by enumeration of g*EC* on the surface of each commodity and mulch coupon. If samples were below the limit of detection, samples were enriched following FDA BAM. Summary statistics and significant differences in g*EC* by drop height and commodity ($p \leq 0.05$) were determined by Tukey's HSD test in RStudio (V4.2.3).

Results: Samples visibly damaged that were not marketable quality were excluded. Cucumber and tomato samples dropped from 4ft (33%; 17%) and 6ft (100%; 43%) were unmarketable, respectively. Significant differences were observed between cucumber log percent transfer of 0ft to all drop heights with marketable samples ($p<0.05$). Significant differences were not observed between jalapeño or tomato log percent transfer and all drop heights with transfer ($p>0.05$). Significantly higher transfer was observed between cucumber to jalapeño across all drop heights with samples ($p<0.05$). *E. coli* concentration was significantly higher in tomatoes dropped on biodegradable mulch compared to plastic mulch for all drop heights ($p<0.05$).

Significance: Regardless of drop height, *E. coli* transferred to dropped produce and should not be harvested due to physical damage and potential contamination.

P3-182 Factors Affecting *E. coli* O157:H7 Proliferation during Sprouting and Postharvest Storage of Alfalfa Sprouts

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Introduction: Developing ways to prevent or minimize pathogen proliferation during sprouting is crucial to further mitigate the public health risks of sprouts. U.S. Food Code defines sprouts as a Time/Temperature Control for Safety food. However, data supporting this designation are lacking.

Purpose: Examine the proliferation of *E. coli* O157:H7 during sprouting and storage of alfalfa sprouts, as affected by temperature, pathogen load and seed treatment.

Methods: One kg of alfalfa seeds inoculated with *E. coli* O157:H7 at a high or low level (4 or 1 log CFU/g) were treated with water or 20,000 ppm $Ca(OCl)_2$ for 15 min. Treated seeds were sprouted in glass jars at 4, 10, 20 or 30°C. Harvested sprouts were stored at 4, 7, 10 or 25°C for 21 days. Samples were taken for analysis of *E. coli* O157:H7 by plate count, 3-tube MPN or culture enrichment.

Results: In seeds contaminated at a high or low level and sprouted at 10/20/30°C, *E. coli* O157:H7 proliferated and reached 6-8 or 4-6 log CFU/g, respectively, but it decreased by up to 3 logs during sprouting at 4°C. Seed treatment reduced *E. coli* O157:H7 on seeds and could prevent pathogen proliferation during sprouting. In seeds inoculated at a low level and treated with $Ca(OCl)_2$, the pathogen did not proliferate during sprouting at 10°C and was not detected (< -1 log CFU/g) in sprouts grown at 4°C. During storage of sprouts grown at 20/30°C, *E. coli* O157:H7 population did not change, regardless of temperature. For sprouts grown at 10°C, pathogen proliferation was not observed during storage at 4/7/10°C, but it could occur in sprouts stored at 25°C. For sprouts grown at 4°C, *E. coli* O157:H7 population remained unchanged or decreased during storage by up to 1.5 logs.

Significance: Knowledge gained will support development of controls that minimize pathogen proliferation during sprouting and inform recommendations made in the Food Code.

P3-183 Application of Vitamin K3 Water-Soluble Analogue as a Wax Supplement for the Control of Foodborne Pathogens on Fruit Surfaces

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❖ *Developing Scientist Entrant*

Introduction: Waxing is a key final step in preparing fruits and vegetables for shipping and storage. Menadione sodium bisulfite (MSB), a vitamin K3 water-soluble analogue, is known for low toxicity and its ability to generate reactive oxygen species (ROS). These qualities suggest MSB as a potential wax additive to improve produce safety.

Purpose: The objective of this study was to investigate MSB's bactericidal effects on fruit surfaces.

Methods: The experiment used unwaxed tomatoes, apples, and oranges. Surfaces were inoculated with *L. monocytogenes* or *Salmonella* Gaminara cultures, dried overnight, and then coated with a commercial wax, with or without 40 mg/mL MSB. Control fruits were sprayed with Milli-Q water. Stored at 22°C, the fruits were sampled over seven days for bacterial enumeration.

Results: Initial *L. monocytogenes* levels on fruits were around 7.7 log CFU/fruit. After 7 days, control and wax-only samples showed *L. monocytogenes* reduction ranging from 1.2 to 1.8 log. In contrast, MSB-treated fruits showed significantly higher reductions (2.4 log in tomatoes, 2.6 in apples, and 3.2 in oranges) ($p<0.05$). MSB was more effective against gram-negative SG. Initial SG levels on fruits were around 8.1 log CFU/fruit. The control samples exhibited minor SG reductions (0.1-0.3 log CFU/fruit), while wax application alone reduced SG by 1.4 to 2.2 log. However, MSB-enhanced wax was more potent, leading to reductions of 3.6 log in tomatoes, 4.0 in apples, and 3.5 in oranges ($p<0.05$).

Significance: Understanding the antimicrobial efficacy of MSB under standard room temperature/light conditions is pivotal in developing a universal bactericidal wax supplement. Using MSB-enhanced wax could lead to ongoing pathogen inactivation in fresh produce, presenting an efficient solution for fruit and vegetable packinghouses.

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P3-184 Mitigation of Cross-Contamination in Hydroponics by Robotic Fertigation

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❖ ***Developing Scientist Entrant***

Introduction: Hydroponics is a promising system to meet the growing demand for fresh produce from a growing population along with heightened interest in urban agriculture; however, cross-contamination risk is inherent within interconnected aqueous systems.

Purpose: Introduction of a robotic fertigation system for delivery of nutrients in a hydroponics may reduce bacterial spread if contamination were to occur.

Methods: Romaine lettuce was grown from seed for 24 days in 4 deep water culture systems. Two of the four systems had recirculating water and two used robotic fertigation delivered hourly. Plant leaves/roots were spot inoculated with ~10^8 log *Escherichia coli* TVS355. Two trials were conducted (n=88). Roots and leaves of inoculated (n=64) and control (n=24) plants were harvested separately per trial at 0, 1, 2, 5, and 7 days post-inoculation (dpi) plus 6 water samples. Universal pre-enrichment broth was added to each bag, plants massaged, and bacteria enumerated on MacConkey+rifampicin. Statistical analysis was completed with One-way Analysis (ANOVA, Means/Std Dev, Quantiles, Comparing Means) in JMP.

Results: *E. coli* inoculated onto roots was internalized from roots to leaves in both systems. Lower average *E. coli* concentrations were detected on control plants across both trials using the robotic fertigation system (p=0.0009). The *E. coli* concentration detected on the leaves of control plants at 1-7 dpi at an average of 1.0 log CFU/g with the robotic system was significantly lower than that for the no robotic system which had an average of 2.8 log CFU/g (p<0.0001). *E. coli* concentrations from leaves and roots in the robotic system were significantly different on 1 and 2 dpi (p=0.0075, p=0.0142) compared to that of 5 and 7 dpi, perhaps associated with increased circulating water volumes.

Significance: Implementation of robotic fertigation in a hydroponic deep-water system resulted in decreased *E. coli* in the leaves of harvested plants.

P3-185 Controlling *Salmonella enterica* in Roots of Indoor-Grown Lettuces

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* has recently been associated with foodborne illnesses, particularly in raw fruits and vegetables, including lettuce grown indoors, which emphasize the importance of implementing thorough agricultural practices and utilizing chemical sanitizers for postharvest washing to combat microbial contamination.

Purpose: This study aims to evaluate the reduction of *Salmonella* from lettuce roots with and without media plugs, employing a no-rinse treatment in comparison with a simulated washing system using 200 ppm chlorine and 80 ppm peroxyacetic acid (PAA).

Methods: Butterhead lettuce "Rex" switched from ebb and flood irrigation to vertical wick irrigation after two weeks. The Salmonella culture (*Salmonella enterica* Newport, Typhimurium and Enteritidis) was adjusted to a 0.5 McFarland scale and diluted to obtain a working culture of 1.5 x 10^8 CFU/ml, and were inoculated onto lettuce roots with and without media plug. Lettuce roots were immersed in the inoculum without agitation for 2 minutes, followed by exposure to UV light for 2 hours. After each treatment, the bacterial reduction in roots-inoculated lettuce was assessed at 0 hours, 24 hours, and 7 days of storage in clam shells at 4°C.

Results: The use of sanitizers had a significant log reduction in the two types of media plug on lettuces and did not show significant differences ($p \geq 0.05$) at 0h, 24h and 7 days for both inoculated roots. The initial concentration for the non-rinse treatment was 5.01 log CFU/g for lettuce without plugs and 6.8 log CFU/g for lettuce with plugs. Within a 24-hour period, lettuce without plugs showed a decrease of 2.40 CFU/g for chlorine and 1.77 CFU/g for PAA compared to a non-rinse treatment. In contrast, when compared to the non-rinse treatment, lettuce with plugs exhibited a reduction of 5.47 and 4.25 CFU/g for chlorine and PAA, respectively.

Significance: These findings imply that lettuce root sanitation may be a useful strategy to control the contamination of whole lettuce against human pathogens.

P3-186 Establishment of Experimental Models Mimicking Natural Contamination to Investigate the Survival of *Escherichia coli* O157:H7 on Walnut Kernels

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Introduction: Tree nuts have been implicated in a number of foodborne outbreaks in recent years including those caused by *Escherichia coli* O157:H7. However, there are lack of models mimicking natural ways of contamination by *E. coli* 0157:H7 to investigate its survival on walnut kernel during long-term storage.

Purpose: This study established two experimental models mimicking natural contamination, namely direct and indirect inoculation models, to investigate the survival of *E. coli* O157:H7 on walnut halves and pieces.

Methods: In the direct inoculation model, the suspension of a single strain of *E. coli* O157:H7 at concentrations from 1 to 10^7 colony forming unit per gram (g) was inoculated into 25 g of walnut kernel. In the indirect inoculation model, walnut kernel (25 g) was mixed with sand (200 g) contaminated with the suspension of a single strain of *E. coli* O157:H7 at the concentration of approximately 17.5 bacterial cells per gram of sand. Walnut halves and pieces contaminated with *E. coli* O157:H7 strain were air dried and stored in sealed bags at either ambient temperature or 25 ºC. The organisms were tested one day, two months and one year after inoculation using both direct plating and enrichment procedures with confirmation by polymerase chain reaction.

Results: For the direct inoculation model, there was a declining of the numbers of the live organisms with the time by using selective plating and enrichment. The organisms could be recovered one year after inoculation for both models with an approximate 3 log reduction.

Significance: This study has established two models mimicking potential natural contamination with *E. coli* O157:H7 for future investigation of the mechanisms of the survival of *E. coli* O157:H7 and other foodborne bacterial pathogens on walnut kernel. The results provide further risk information about the persistence of *E. coli* O157:H7 on walnut kernel.

P3-187 Use of Biological Soil Amendments of Animal Origin on Fresh Produce Farms

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Introduction: The FSMA Produce Safety Rule inspection covers the use of biological soil amendments of animal origin (BSAAO). Understanding the frequency and methods of BSAAO use on farms growing fresh produce is necessary to provide educational resources, inform research, and offer guidance.

Purpose: To demonstrate the use of BSAAOs on fresh produce farms in the United States, including the type of BSAAO, the method of application, the crops grown, and if the BSAAO is treated.

Methods: An anonymous survey was distributed online via Qualtrics™ between September 2023 and January 2024 to the produce industry nationally. Grower responses were used to identify current industry trends with BSAAO use. Descriptive statistics were used to assess percentage of responses.

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Results: Thirty-six growers responded to the survey, representing 50% (38/76) of the total survey responses. Growers were very or somewhat concerned (53% 20/38) about food safety risks associated with BSAAO used to grow fresh produce. Of the grower respondents 63% use BSAAO (24/38). Poultry litter (54% 13/24), compost (50% 12/24), blood meal (46% 11/24), and bovine manure (42% 10/24) were the most highly indicated BSAAOs used. BSAAOs were applied through field spreading (63% 15/24), foliar sprays (21% 5/24), soil injections (17% 4/24), and irrigation systems (17% 4/24). The most common crops the BSAAOs were used on were leafy greens (46% 11/24), brassicas (38% 9/24), legumes (38% 9/24), and cucurbits (33% 8/24). The following BSAAOs had the highest frequency of responses showing use without treatment or being unsure of treatment: bovine manure 100% (10/10), poultry litter 85% (11/13), blood meal 66% (7/11), and compost 66% (8/12).

Significance: Educational outreach is needed to assist growers in meeting the FSMA Produce Safety Rule requirements. Educators should focus on risk reduction methods, proper treatment methods, and how to verify purchased treated BSAAOs.

P3-188 Inhibition of Shiga Toxin-producing *Escherichia coli* Strain MD41 by *Pseudomonas fluorescens* Strain A506 on Romaine Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: Contamination of leafy vegetables with Shiga toxin-producing *E. coli* (STEC) poses a challenge to the horticultural industry and public health. Biological control agents may be useful as a hurdle technology in controlling contamination of leafy vegetables with enteric pathogens, however their mode of action must be better understood.

Purpose: This study aims to investigate the effect of the well-known biocontrol agent *Pseudomonas fluorescens* strain A506 on the colonization of the romaine lettuce phyllosphere by STEC strain MD41.

Methods: Middle romaine lettuce leaves from plants grown in the laboratory for eight weeks were pre-inoculated with A506, its mutant A506-1, which is deficient in pyoverdine siderophore production, or potassium phosphate buffer. After a 24-h incubation of the leaves, MD41 was introduced onto the leaves at inoculation levels ranging from 3 to 6 log CFU/g leaf tissue, and the leaves were incubated at 28 °C for up to 72 h. Each group included six replicates, and Tukey's HSD test was conducted for multiple comparisons.

Results: The cell viability of MD41 in spent supernatant of A506 cultured in Luria-Bertani broth did not decrease compared with the control, as revealed by the Live/Dead assay. Pre-inoculation with A506 or A506-1 suppressed MD41 population growth over 36 h when the enteric pathogen was inoculated at low levels, with the siderophore-positive strain A506 exerting stronger inhibition. At higher MD41 inoculation levels, the population declined ca. 10-fold after pre-colonization of the leaves with A506 compared to the control, but not significantly with A506-1.

Significance: These findings indicate that low iron availability to STEC due to siderophore-mediated iron depletion by A506 may contribute to the inhibitory effect of this biocontrol strain on the enteric pathogen on lettuce leaves. Our study emphasizes the role that competitive interactions between the phyllosphere resident microbiome and immigrant enteric pathogens may play in the outcome of contamination events and the potential of exploiting such interactions to improve produce safety.

P3-189 Investigating the Antimicrobial Effect of Different Post-harvest washing Solutions on Spinach and Tomatoes

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Introduction: Post-harvest washing is a critical step to minimize the microbial contamination of fresh produce. It is important to apply an effective washing solution to achieve a high bacterial reduction while maintaining the original color and texture of fresh produce.

Purpose: This study aimed to evaluate the antimicrobial efficacy of three washing solutions (water, 100 ppm sodium hypochlorite, 1% w/v citric acid) at various parameters and their effect on the color and texture of spinach and tomatoes.

Methods: Spinach and tomatoes were spot inoculated with *E. coli* (10^7 CFU/ml), respectively. The inoculated samples were washed separately with the three washing solutions. Various washing parameters were tested including washing time, temperature, and agitation condition. After each washing treatment, samples were plated on Trypticase soy agar (TSA) to determine the bacterial reduction. Another set of samples was stored for 7 days after washing. At a 2-day interval, samples were analyzed to determine the antimicrobial effect during the storage. All samples were also evaluated for color with a colorimeter and texture with a texture analyzer after each treatment. Three biological replicates and three samples per replicate were used for each treatment (n=9).

Results: Washing time and temperature did not significantly ($p>0.05$) affect the antimicrobial efficacy of three washing solutions. Washing with agitation resulted in a higher bacterial reduction than that without agitation. Citric acid (1%) resulted in a higher bacterial reduction ($p\leq0.05$) for immediate washing and during storage than the other two washing solutions. There was no significant effect ($p>0.05$) on color and texture for all three washing solutions.

Significance: The study demonstrated higher antimicrobial efficacy of 1% citric acid for immediate washing and during storage without a negative impact on the color and texture of fresh produce and provided a better understanding of the effect of various washing parameters and food matrices.

P3-190 *Escherichia coli* O157:H7 Strains Associated with Reoccurring Lettuce Outbreaks Display Strong Biofilm-Forming Ability and Low Sanitizer Susceptibility

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Introduction: Genetically closely related E. coli O157:H7 strains were responsible for several recent multistate outbreaks linked to romaine lettuce. But the factors that contributed to their reoccurrence and persistence remain elusive.

Purpose: Biofilm formation plays an important role in the environmental persistence of foodborne pathogens. The objective of this study was to investigate the biofilm-forming capability of the outbreak strains and their biofilm resistance to antimicrobials.

Methods: Seven strains, three from recent lettuce outbreaks, and four from previous lettuce, spinach, and hamburger outbreaks, were included in this study. Biofilm formation was investigated by immersing the stainless-steel coupons into inoculated M9 medium,10% tryptic soy broth (TSB), and 10% lettuce juice (LJ) at 25 °C for 48 h. Biofilm density was evaluated and the formed biofilms were treated with 200 ppm of quaternary ammonium compounds (QACs) for 1 min. Biofilm architecture and cellulose production were visualized with the aid of fluorescent dyes under a CLSM.

Results: The recent outbreak strains achieved higher biofilm density (8.07 log CFU/cm^2) in M9 medium than the other strains (6.46 log CFU/cm^2), but no significant difference ($p\geq0.05$) was observed in 10%TSB and 10%LJ. Biofilms formed by the recent outbreak strains in M9 medium, and 10%TSB were extremely resistant to QACs treatment, with log reductions of 0.03 and 0.55, respectively. However, QACs resistance of biofilms formed in 10%LJ was not significantly different. The microscopic analysis showed that the recent lettuce outbreak strain formed more compact and thicker biofilms with higher cellulose content, while small clusters or individual cells with less cellulose production were observed in the other strains. The complex biofilm structure and the thicker layer of cellulose might explain their stronger QACs resistance.

Significance: The results of this study would provide insight into why these strains are persistent and how to prevent them in the future.

P3-191 Characterization of an ESBL-Producing Strain of a New *Enterobacter* Species from Malabar Spinach

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Introduction: Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales* are considered a serious threat by CDC. *Enterobacter* species, belonging to *Enterobacterales*, are an increasing cause of nosocomial infections. Fresh vegetables may serve as reservoirs for antibiotic-resistant bacteria, including ESBL-producing *Enterobacterales*.

Purpose: The study aimed to characterize an ESBL-producing *Enterobacter* strain from a retail Malabar spinach sample.

Methods: In the process of isolating ESBL-producing *Enterobacterales* from fresh vegetables, CHROMagar™ ESBL was used to isolate ESBL-producing *Enterobacterales* from a Malabar spinach sample after pre-enrichment in buffered peptone water and enrichment in Enterobacteria Enrichment Broth. ESBL and AmpC β-lactamase production of isolates was confirmed using combination disk diffusion assays. The genome of one isolate was sequenced by both Illumina and Nanopore sequencing. Antibiotic resistance (AR) genes carried by the strain were identified using the Resistance Gene Identifier program. Ribosomal Multilocus Sequence Typing, digital DNA-DNA hybridization (dDDH), and Average nucleotide identity (ANIb) were used to determine the taxonomic position of the strain.

Results: One ESBL- and AmpC β-lactamase-producing isolate 008-E1 was obtained from a local Malabar spinach sample and sequenced. The strain carried an ESBL gene bla_{FONA-6} on a plasmid and an AmpC β-lactamase gene bla_{ACT-29} on the chromosome. The strain was identified as an *Enterobacter* species. However, the highest dDDH value between strain 008-E1 and type strains of other known *Enterobacter* species was 50.0%, below the 70% cut-off, and the highest ANIb value between strain 008-E1 and type strains of other known *Enterobacter* species was 92.6%, below the 95% cut-off, indicating that 008-E1 is a new species of the genus *Enterobacter*.

Significance: Fresh vegetables can harbor undiscovered species of antibiotic-resistant bacteria, and a reservoir for ESBL-producing *Enterobacterales* carrying AR genes in transferable elements. The results highlight the necessity of AR mitigation in fresh vegetables.

P3-192 Effects of Biotic and Abiotic Factors on Attachment and Survival of *Salmonella* Typhimurium on Post-Harvest Produce

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Introduction: Salmonella contamination of post-harvest produce is a persistent produce safety problem worldwide. Quantifying factors that limit pathogen attachment /survival is essential for consumer safety.

Purpose*:* Determine factors that influence and adversely mitigate Salmonella Typhimurium attachment and its survival on produce.

Methods: Post-harvest carrots, alfalfa sprouts, soybeans and pistachio were inoculated with 6-7 log CFU/g of Salmonella Typhimurium by dipping produce in 20 mL of bacteria suspension in sterile stomacher bags. Storage temperature (5 and 25C) effects on Salmonella attachment (SR-values) were quantified after inoculations. Treatments of pathogen-inoculated produce with a biosurfactant (lauric acid amide pyrrolidine, LAPY), bacterial competitor (Bacillus subtilis), and physical decontamination by peptone water (PW) rinse on Salmonella attachment and survival were determined. SEM imaging was done to compare Salmonella attachment on produce before and after treatments. Pathogen populations were quantified by plating 100µL aliquots from rinses and dilutions from stomached produce on XLT-4 selective medium (37C). Data on Salmonella populations were converted to Log CFU/g and were analyzed with Analysis of Variance (ANOVA) to assess significance of treatment effects (Statistical Analysis System (SAS) Institute, Cary, NC).

Results*:* Storage temperatures influenced the strength of Salmonella attachment on produce types as S_R-values were significantly greater (p<0.05) on pistachio and soybean (0.53-0.76), than on alfalfa and carrots (0.10-0.13). LAPY application resulted in greater pathogen reductions on alfalfa, pistachio and soybean (2.1-3.0), but not on carrot (<1.5 log). The presence of a bacterial competitor (*B. subtilis*) on produce in combination with S. Typhimurium resulted in non-significant reductions in pathogen attachment, except on alfalfa sprouts. Physical decontamination of pathogen-inoculated produce by PW rinse significantly (p<0.05) reduced bacterial attachment and Salmonella populations on produce.

Significance: These results indicate that mitigation of *Salmonella* attachment by selective application of post-harvest decontamination measures may limit pathogen survival and enhance the safety of post-harvest produce.

P3-193 Assessment of Vermicompost Compositions Containing Cattle, Sheep, and Poultry Manures for Contamination Risk of Microgreens

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Introduction: Transfer of pathogens to microgreens may occur due to contamination of the seed, the growing medium, irrigation water, and equipment.

Purpose: The aim of this study was to assess solid and liquid vermicompost compositions containing cattle, sheep, and poultry manures for contamination risk of microgreens.

Methods: Pasteurized vermicompost compositions were produced with composted cattle, sheep, and poultry feces. Liquid vermicompost (tea) was prepared by filtering 300 g of solid vermicompost compositions and 1 l of sterile distilled water mixture. Ampicillin (Amp) resistant 3-strain cocktails of *Escherichia coli* O157:H7 (STEC) and *Salmonella* and non-resistant *Listeria monocytogenes* were inoculated (10^5-10^7 CFU/g) into solid vermicompost and prepared vermicompost tea diluted with distilled water (1:1) before use. Inoculated vermicomposts were stored at room temperature. Bacterial populations were enumerated for up to 28 days on selective media. Radish microgreens were grown in vermicompost tea-soaked perlite as soilless substrate. After harvesting, the population of transferred pathogens were enumerated (n=4). SMAC and XLD supplemented with Amp (100 µg/ml) and PALCAM were used for STEC, *Salmonella*, and *L. monocytogenes*, respectively.

Results: Pathogen persistence was not affected by vermicompost compositions and prepared vermicompost teas made of different animal manures (p>0.05). Similar survival trends were observed for STEC and *Salmonella* in solid vermicompost compositions and vermicompost tea derivatives. Over the 28-day storage, STEC and *Salmonella* populations declined between 4.5 and 5.7 log CFU/g in solid vermicompost compositions, and 3.9 and 5.9 log CFU/g in prepared vermicompost teas, respectively (p≤0.05). Reduction in *L. monocytogenes* populations were ≤1.8 log CFU/g in all vermicompost preparations. Pathogen populations transferred to microgreens from vermicompost tea-soaked perlite ranged from 5.6±1.0 to 7.1±0.1 log CFU/g.

Significance: The use of contaminated or inadequately pasteurized solid vermicompost and derived vermicompost teas made of animal feces could pose a high risk for contamination of microgreens if not handled and managed properly.

P3-194 Effectiveness of Sanitizers Commonly Used by the Greenhouse Industry to Eliminate *L. monocytogenes* from Hydroponic Surfaces Used in Deep Water Culture Systems

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Introduction: Regular cleaning and sanitation of hydroponic systems is critical to ensure the safety of hydroponic crops. There are currently no validated protocols for sanitation of Deep-Water Culture (DWC) hydroponic production surfaces.

Purpose: To evaluate the effectiveness of sanitizers in eliminating *Listeria monocytogenes* from DWC hydroponic surfaces including floaters made from high-density polyethylene (HDPE) and polystyrene, Oasis dry foam, and HDPE liner.

Methods: All surfaces were cut into coupons and inoculated with ~10^7 CFU/cm^2 *L. monocytogenes* (100mg/lit nal). After drying, the coupons were treated

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with sanitizers (Na-hypochlorite (100 and 200 ppm), SaniDate 12.0 (100 and 200 ppm), Virkon (1%), Kleengrow (2%), hydrogen peroxide (3%)) commonly used in greenhouses, for a contact time of per manufacturer label (5-10 min). Surfaces were processed, and serial dilutions were enumerated on LB agar after incubation at 37°C for 24h. Mean log reductions were calculated.

Results: Na-hypochlorite (100 and 200 ppm) was not effective in eliminating *L. monocytogenes* from DWC surfaces and achieved less than 3 log reduction on most surfaces: HDPE liner (2.20±1.19 and 2.05±0.61 log CFU/cm^2, respectively), OasisÒ (2.74±0.36 and 3.45±0.90), HDPE floater 2.17±1.79; 2.12±0.76 CFU/cm^2, respectively), and Styrofoam floater (1.38±0.11; 1.96±0.54 CFU/cm^2 respectively). In fact, Na-hypochlorite treatments were similar to water control on the surfaces of liner (p=0.1) and floater (p=0.3). SaniDate, Virkon, Kleengrow and hydrogen-peroxide eliminated *L. monocytogenes* from all four surfaces achieving > 5 log reduction on all surfaces.

Significance: The findings from this study provide hydroponic growers with information about effectiveness of sanitizers that can help them chose appropriate treatments and improve food safety of hydroponic produce.

P3-195 Foliar Application of a Novel Ascaroside Reduces *Escherichia coli* O157:H7 on Romaine Lettuce

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Introduction: There are few effective ways to reduce fresh produce contamination by foodborne pathogens after harvesting. However, pre-harvest treatments that stimulate the plant's immune response may be a possible solution. One such treatment involves using the nematode-derived molecule ascaroside. This treatment has the potential to offer protection against both pre-and post-harvest contamination of fresh produce.

Purpose*:* This study seeks to understand the efficacy of Novel Ascaroside#1 (NA1) treatment on lettuce contaminated with *Escherichia coli* O157:H7.

Methods*:* NA1-based treatments were applied to romaine lettuce via foliar spray at different concentrations (Extra- low-0.01µM, low-0.1 µM, high-1 µM) three times. Each head received 3 mL of the corresponding formulation. The first treatment was given four weeks after planting, followed by the second and third treatments two days before and after inoculation. A four-strain cocktail of rifampicin-resistant *E. coli* O157:H7 (CDC 658, K3995, F4546, H1730) was spot inoculated on the lettuce heads (c. 5 logs CFU/mL) and allowed to dry for 1 hour. On day 7, inoculated lettuce heads were washed with 250 ml of 0.1% peptone and Tween-80, and rinsate was plated in Tryptic Soy Agar after serial dilution. When populations were below the plating limit of detection, the most probable number (MPN) method was used. Statistical analyses were performed in R.

Results*:* The results of a one-way ANOVA analysis of MPN *E. coli* O157:H7 per head of lettuce showed no significant differences 7 days after inoculation: $F(3, 14)=0.468$, p=0.70. The extra-low, low, and high treatments resulted in reductions of 1.04, 0.83, and 0.81 log CFU/head respectively, compared to the no-treatment controls.

Significance: While the preliminary data indicates no significant difference among the treatments and controls, alterations in application method and concentration may result in more significant findings. Further studies may be needed to optimize the application of this treatment.

P3-196 Pulsed Light and Sanitizer Wash Combination Enhances Inactivation Efficacy Against *Escherichia coli* O157:H7 in Romaine Lettuce

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Introduction: Romaine lettuce has been frequently implicated in outbreaks of foodborne illness. The produce industry uses chlorine-based sanitizer wash which has only limited efficacy. Safe and effective new methods are needed.

Purpose*:* The purpose of this study was to investigate the efficacy of integrated treatment of Pulsed light (PL) and sanitizer wash (SW) against *E. coli* O157:H7 on Romaine lettuce, as an effective alternative strategy to current chlorine wash.

Methods*:* Romaine lettuce was spot inoculated with a bacterial cocktail containing three outbreak strains of *Escherichia coli* O157:H7 to a level of about 6 log CFU/g. Two different combination treatment strategies were explored, namely, PL followed by SW (PL-SW) and SW followed by PL (SW-PL). Briefly, Romaine lettuce was washed with 250 mL sanitizer for 2 min before and after PL treatment for 10 s (10.5 J/cm^2). Homogenates of lettuce sample were prepared by stomaching in peptone water. Pathogen populations were quantified by plating 100 µL of homogenate dilutions on SMAC selective medium (37°C). When populations were below detection limit, pathogen absence in the sample was confirmed by enrichment methods. Experiments were conducted in triplicate and data were analyzed with ANOVA to assess significance of treatment effects by statistical analysis system (SAS Institute, Cary, NC).

Results*:* Statistically significant (p<0.05) increases in pathogen inactivation was achieved by combination treatments of PL and SW compared to individual treatments. PL (10 s) and SW (2 min) treatments individually inactivated 2.3±0.32 and 2.2±0.25 logs of *E. coli,* respectively on Romaine lettuce. Both combination treatments (PL-SW and SW-PL) resulted in synergistic inactivation as *E. coli* cells were not detectable after combination treatments, indicating >5 log pathogen reductions.

Significance: These results suggest that PL and SW combination is an efficacious treatment strategy which can ensure the microbial safety of Romaine lettuce.

P3-197 Microbial Population Distinctions between Open Field Grown Versus Controlled Environmental Agriculture Grown Leafy Greens

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❖ ***Developing Scientist Entrant***

Introduction: Growth conditions could influence the microbiota of plants. Lactic acid bacteria (LAB), considered as probiotics, are beneficial to human health. Controlled Environmental Agriculture (CEA) production of leafy greens (LGs) is a rapidly expanding sector of agriculture providing year-around production. Comparative differences in the microbiota of CEA produce compared to open field-grown (OFG) produce are unknown for either total microbial populations or beneficial microbes such as LAB.

Purpose: Evaluate and compare the total microbial populations and LAB populations on different types of LGs grown in open fields as well as in CEA.

Methods: Samples were collected from OFG LGs (chard, romaine hearts, spinach, arugula, and chopped romaine) and from CEA-grown LGs (chard, romaine hearts, frisee lettuce, red leaf lettuce, and green leaf lettuce) and analyzed. Total microbial populations and LAB populations were enumerated by serially diluting stomached samples, then spread-plating onto Tryptic Soy agar and *Lactobacillu*s de Man Rogosa and Sharpe (MRS) agar, respectively. Differences in native microbiota populations and LAB populations among LGs from OFG and CEA-grown conditions were analyzed.

Results: The OFG LGs had significantly higher total microbial populations (p<0.05) than CEA-grown LGs. Although LAB populations in general from all five CEA- and OFG LGs sources weren't significantly different (p>0.05), CEA had a higher overall percentage of LAB populations. A paired comparison between OFG and CEA-grown chard and romaine hearts (common in both agricultural conditions) showed that OFG chard and romaine hearts had significantly higher total microbial populations (p<0.05) than CEA-grown. CEA-grown chard and romaine hearts had significantly higher LAB populations (p<0.05) than OFG.

Significance: CEA-grown LGs harbored significantly lower (p<0.05) total microbial populations than OFG. From a food safety perspective, this could indicate a reduced risk of exposure to foodborne pathogens from CEA-grown LGs. Results also indicated that CEA-grown LGs may be a good source of probiotics in comparison to field-grown LGs.

P3-198 Effect of Netting Density on the Efficacy of Postharvest Sanitizers in Reducing Foodborne Pathogenic Bacteria on Cantaloupe Rinds

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Introduction: The increase in cantaloupe consumption has been associated with the incidence of several multistate foodborne outbreaks. Since cantaloupes grow on the ground, direct soil contact represents a potential risk for the consumers if the rinds become contaminated with foodborne pathogens.
Purpose: This study evaluated how the density of rind netting affects the efficacy of postharvest sanitizer in reducing foodborne pathogenic bacteria from cantaloupe surfaces.
Methods: Three cantaloupe varieties with different rind characteristics (smooth-, medium-, and heavy-netted cantaloupes) were inoculated with a mixed culture containing pathogenic strains of *Salmonella* Typhimurium, *E. coli* O157:H7, and *Listeria monocytogenes.* Each treatment consisted of immersion of the rectangular shaped cantaloupe rinds portions (~35cm^2, 25g) in 2L of sanitizer solution for 5min. Sanitizers included sodium hypochlorite (as measured by free available chlorine; 200mg/L), peracetic acid (80mg/L), chlorine dioxide (5mg/L), and distilled water alone (for water control). Individual samples were placed in a sterile blender machine and blended in 225ml of neutralizing solution for 1min. The obtained solution was plated on their respectively selective media. Full factorial analysis was implemented (p<0.05).
Results: It was observed that as the density of the netting increased, the efficacy of the sanitizers on the reduction of foodborne pathogenic bacteria decreased (p<0.05). Specifically, bacterial reduction was higher in the smooth-rind variety when PAA and FAC were applied, in contrast to the reduction obtained after treating heavy netted cantaloupes (p<0.05). While chlorine dioxide contributed to the reduction of the load of *E. coli, Salmonella,* and *Listeria,* its efficacy was significantly lower than the obtained by PPA and FAC treatments (p<0.05).
Significance: These findings highlight that the netting on cantaloupe rinds has the capacity to harbor and protect pathogenic bacteria from the action of postharvest sanitizers. Contributing to the development of appropriate interventions to improve cantaloupe safety and postharvest handling.

P3-199 Antimicrobial Susceptibility of Microbiota Associated with *Spinacea oleracea* var. capitata and *Brassica oleracea* L. from Farms and Retails

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Introduction: Leafy green vegetables are a highly variable group of perishable food that broadly can be defined as vegetables grown for their edible leaves. The emergence of various antibiotic resistant bacteria particularly in the food chain has become a major area of concern.
Purpose: This study aimed to evaluate the antimicrobial susceptibility of foodborne pathogens isolated from leafy greens at the farms and retails.
Methods: Sixteen isolates were analyzed for antimicrobial susceptibility test against various antibiotic agent. Samples were streaked onto Mueller hinton agar plates and incubated at 37°C for 24h. Target organisms were *E. coli,* coagulase-positive *Staphylococci, Listeria* and *Bacillus.* Using Kirby Bauer disc diffusion technique the isolates were subjected to a panel of nine antimicrobial agents: penicillin (P) (10_µg), ampicillin (AMP) (10_µg), gentamicin (CN) (10_µg), ceftazidime (CAZ) (30_µg), chloramphenicol (C) (30_ µg), tetracycline (TE) (30_µg), vancomycin (VA) (30_µg), erythromycin (E) (15_ µg) and ciprofloxacin (CIP) (5_ µg).

Results: Generally, thirteen isolates (13/16; 81.25%) were susceptible to vancomycin and only three isolates (3/16; 19%) were resistant. Twelve isolates (12/16; 75%) were susceptible to gentamycin and four (4/16; 25%) were resistant. Ten isolates (10/16; 63%) were susceptible to chloramphenicol and tetracycline, four isolates were resistant (4/16; 25%) and two isolates were intermediate (2/16; 13%).
Significance: Leafy greens at the farm and retail level in South Africa may assume an important role in the maintenance and dissemination of antimicrobial resistant pathogens.

P3-200 Effect of Cow Manure Compost on Microbial Quality of Strawberries

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Introduction: Manure-based biological soil amendments (MBBSA) are applied to enhance crop growth and improve soil quality and health. However, due to the potential presence of naturally occurring foodborne pathogens (FBP) originating from livestock intestine, the application of MBBSA may result in an increased risk of contamination of fresh produce with FBP.
Purpose: The objective of this study was to assess the microbiological quality of strawberry in response to MBBSA.
Methods: Strawberry plants were grown in raised beds, covered with black plastic in November. 100 g of commercial cow manure compost was added and covered with one inch of natural soil below the plant root. The trial included three replications of two treatments (cow manure compost and non-amended control), with 20 plants in each replication. In April, ten strawberry samples, each weighing 50 g, were harvested from each plot. Samples were mixed with 0.1% peptone in two-chamber filter bags and blended, and serial dilutions were prepared from the filtrate. The wash filtrates were analyzed for microbiological quality by plating the serially diluted samples onto non-selective and selective chromogenic agar plates to test for the presence of total aerobic bacteria, Enterobacteriaceae, *Escherichia coli* and coliforms, *E. coli* O157, *Salmonella*, and *Listeria*. The microbial community structure was also analyzed by extracting bacterial genomic DNA from the fruit wash filtrates and sequencing the V4 region of the bacterial 16S rRNA gene.
Results: Alpha-diversity significantly increased in strawberries grown in cow manure-treated compared to untreated control soils ($p \leq 0.05$). The PCoA of Weighted UniFrac distance showed a distinct separation between strawberries grown in treated soils and those in untreated control soils. Populations of *Proteobacteria*, including *Gammaproteobacteria*, were significantly enriched in strawberries grown with cow manure. However, potential foodborne pathogens such as *Escherichia coli*, *Salmonella* and *Listeria*, were not detected.
Significance: Properly composted manure does not increase the risk of contamination with foodborne pathogens.

P3-201 Effect of Untreated Biological Soil Amendments of Animal Origin on the Microbial Food Safety Risk of Bell Peppers (*Capsicum annuum*)

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❖ ***Developing Scientist Entrant***

Introduction: Current food safety rules and standards provide restrictions on the use of untreated Biological Soil Amendments of Animal Origin (BSAAOs) for crops that are consumed raw.
Purpose: This study evaluated microbial safety of bell peppers (variety *Turnpike*) grown in fields applied with poultry litter or raw cow manure.
Methods: The field experiment was established with 36 plots (88 ft^2) planted with bell pepper seedlings on 12-inch centers for a total of 20 plants/plot. BSAAO treatments consisted of either raw cow manure (CM), poultry litter (PL), or non-BSAAO (NB) application, with each treatment being either tilled or top dressed (no-till). *E. coli*/Total Coliforms were determined from soil samples collected during weeks 1, 3, 6, and 12 and from harvested bell peppers during weeks 13 and 14. Nalidixic acid resistant *E. coli* was inoculated on bell peppers for a 5-day die-off determination (40 inoculated on the upper portion and 40 on the side).

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Results: Plots with BSAAO application had higher levels of *E. coli* (1.1–2.6 log CFU/g) and coliforms (4.1–4.7 log CFU/g) as compared to NB plots (1.00–1.17 log CFU/g; 3.89–4.14 respectively) *E. coli*/Total Coliforms levels were significantly reduced after a week for plots containing BSAAOs. Bell peppers from no-till CM plots had significantly higher *E. coli* levels, 0.02 log CFU/ cm^2. Within 4 days, *E. coli* levels on bell peppers were reduced by >2 log with a die-off rate of 0.57 and 0.51 log CFU/cm^2/day for bell peppers inoculated on top and on the side, respectively. BSAAOs had no effect on the quality of the bell pepper based on marketable and non-marketable yields.

Significance: Application of untreated BSAAOs can increase the risk of cross-contamination. Environmental conditions affect the survival of *E. coli*, indicating that waiting time between harvest and potential contamination events is a best practice to minimize food safety risks.

P3-202 Controlling *Salmonella enterica* in Water Recirculating Systems for Lettuce Production using a Bacteriophage Cocktail

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Introduction: Controlled environment agriculture has gained popularity in recent years, especially for hydroponics, vertical farms, and aquaponics, in which plants develop without soil using nutrient solutions and substrates. However, concerns have emerged regarding food safety, requiring new strategies to mitigate risks of produce contamination.

Purpose: This study investigates the efficacy of a bacteriophage cocktail as a biocontrol agent against *Salmonella* contamination in lettuce under water recirculating systems.

Methods: *Salmonella* Newport and *Salmonella* Typhimurium (10^3 CFU/mL) were inoculated into each aquaponic and hydroponic nutrient solution to simulate sporadic contamination following a phage cocktail treatment (S7, S10, and S13) at different multiplicity of infection (MOI 0.01 and MOI 1).

***Results*:** Results showed a significant reduction in *S.* Newport and *S.* Typhimurium populations in both MOI 0.01 and MOI 1 in aquaponic and hydroponic nutrient solutions compared to the control, reaching values below the limit of detection (LOD) after 3 to 4 days post-bacteriophage cocktail inoculation. From the plant parts, the microbial population of *Salmonella* serovars in plugs and roots from the hydroponic nutrient solution had a significant reduction, reaching levels below the LOD in both phage cocktail treatments after 2-day inoculation. Contrarily, there were not significant reductions in *S.* Newport and *S.* Typhimurium levels in plant roots and media plugs from the aquaponic nutrient solution treatment compared to the control.

Significance: These findings highlight the potential of using bacteriophage as a tool to improve food safety in indoor-grown lettuce by controlling *Salmonella* populations and the need for future research to understand the microbial interactions within each type of system.

P3-203 An Overview of On-Farm Investigations Associated with Outbreaks of Shiga Toxin-Producing *Escherichia coli* Infections Linked to Leafy Greens: 2009 – 2021

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Introduction: Leafy greens (LG) are a reoccurring source of Shiga toxin-producing *Escherichia coli* (STEC) outbreaks.

Purpose: We present findings from on-farm investigations from STEC outbreaks linked to LG, conducted by the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and state and local partners, during 2009–2021.

Methods: Data on multi-state STEC outbreaks linked to LG were collected during 2009–2021 from CDC's Foodborne Disease Outbreak Surveillance System and FDA's Coordinated Outbreak Response and Evaluation Network. On-farm investigation information was retrieved from FDA Farm Inspection Questionnaires and Investigation Memos.

Results: During 2009–2021 there were 49 LG STEC outbreaks; 37 were linked to a specific LG type. FDA performed traceback investigations for 25, and FDA, CDC, and state partners performed on-farm investigations for 17 outbreaks. Seven outbreaks were traced back to a single growing location (4 in California, 2 Arizona, 1 Nevada) and 10 were traced back to multiple growing locations (7 in California, 1 Arizona, 1 Arizona and California, 1 Oregon and California). An increasing number of samples were collected as years went by, including product (11 investigations), air (3), water (11), sediment (10), scat or manure (9), and soil (10). The outbreak strain was identified in 5 of 17 total investigations. For 7 single farm investigations, 5 farms irrigated with surface water, 1 used well water exclusively, and 1 used both well and surface water.

Significance: Collaboration between FDA, CDC, and local and state partners during on-farm investigations at LG growing locations improved the collection of samples and valuable data, helping identify possible outbreak sources, which can inform prevention efforts. Improved traceability in the supply chain, continued grower education and outreach, adoption of regulations and best practices, and research to address food safety gaps are necessary to reduce the frequency and scope of future outbreaks.

P3-204 Microbial Analysis of Cantaloupe Surfaces Post-Commercial Washing

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✦ ***Undergraduate Student Award Entrant***

Introduction: Recent *Salmonella* outbreaks linked to fresh cut cantaloupes have prompted an investigation into cantaloupe post-washing microbial communities. Despite cantaloupes growing on the ground and being harvested by hand or harvester, the entire fruit undergoes commercial washing, raising questions about microbial loads on different surfaces.

Purpose: The purpose of this study was to gather information about microbial communities on the surface of cantaloupes and to assess whether there are any differences in microbial loads between the top and bottom sides of melons after post-washing on the shelf.

Methods: This study, based on fifteen melons from a local store, employed two microbial sampling methods: surface cloth-rubbing with FREMONTA's MicroTally® Mini Surface Sampler (n=5) and rinsing (n=5). Additionally, a top and bottom comparison used the Mini Surface Sampler (n=5), resulting in 10 samples. Standard microbiological testing methods were used to obtain viable and culturable microbial count results.

Results: Results indicated average aerobic plate counts of 7.25-7.79 logs, total coliforms 2.57-3.12 logs, *Enterobacteriaceae* 2.76-3.26 logs, yeast 5.38-5.48 logs, lactic acid bacteria 2.94-3.25 logs, presumptive *Bacillus cereus* 3.82-4.02 logs, and *Pseudomonas* 4.58-4.62 logs on the overall melon surface. Notably, no *E. coli* and *Salmonella* were detected. While no significant difference was observed between the MicroTally Mini and rinse methods, individual melon variations were apparent, with one melon displaying notably higher APC counts and others having elevated counts in specific microbial groups. Top and bottom comparison revealed minimal differences; two melons exhibited lower microbial loads on the top side, with a difference of 0.06 logs and 1.46 logs, while three melons had higher counts on the top side. Overall, despite individual variations, differences between the top and bottom sides were not significant.

Significance: This study contributes valuable insights into the microbial landscape of cantaloupes post-commercial washing, offering relevant information for food safety considerations.

P3-205 Assessment of Colonization Potential and Microbiome Analyses of Artificially Contaminated Bulb Onions with *Salmonella* newport during Growth and Curing in Controlled Environment Agriculture

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Introduction: A recent multistate outbreak in the USA linked to bulb onions and *Salmonella* Newport sickened 1127 people of which 167 people were hospitalized. However, there is limited knowledge in the contamination potential of *Salmonella spp.* during pre- and postharvest of bulb onion production.

Purpose: The aim of the study was to assess colonization potential of *S.* Newport and microbiome analyses of bulb onions and soil during growth and curing.

Methods: Seed was planted using 45g topsoil and placed into a CONVIRON GEN1000 growth chamber (Temperature 23°C; Humidity 65%). During transplants, ~6120g aliquots of topsoil were weighed and placed into each of 12 pots. Soil was contaminated with three different concentrations of *Salmonella* Newport [10^2 CFU (low), 10^4(medium), 10^6 (high) CFU/200g soil] in triplicate during transplant and two weeks before harvest, with three pots serving as uncontaminated controls. A total of 12 onions were grown, one in each pot. *Salmonella* was detected using the soak method after one week of curing of onions and soil samples. Microbiome analyses was performed on high inoculum samples at 0hr,1hr and 24hr after enrichment in modified buffered peptone water by 16S metagenomic approach.

Results: The size of the grown bulbs onions were different and ranged between 54g to 270g**.** One bulb onion was positive for *Salmonella* in the high inoculum group whereas none of the bulb onions were positive in the low and medium inoculum groups. Two soil samples from each high and medium inoculum group were positive for *Salmonella* while none were positive for the low inoculum group. Microbiome analyses showed that soil and onion microbiome was similar after 24hr enrichment while they were different at 0hr and 1hr.

Significance: This study will be beneficial to understand colonization capability and changes in bacterial community in soil and bulb onions during S. Newport contamination.

P3-206 Matrix Validation of 450 g Romaine Lettuce for the Detection of *E. coli* O157:H7 and *Salmonella* Using Hygiena's BAX® System

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Introduction: Leafy greens, like romaine lettuce, are at high risk for foodborne illness outbreaks. Current industry standards for pathogen analysis are to test a 375 g sample; however, due to the constant hazards, larger quantities are being considered.

Purpose: Rapid methods being used for pathogen analysis must be capable of detecting very low numbers of cells regardless of sample size. To accommodate further testing requirements being added by LGMA, this study evaluated 450 g samples of romaine lettuce with two real-time PCR assays for *E. coli* O157:H7 and *Salmonella*.

Methods: Romaine lettuce was dual inoculated with *E. coli* O157:H7 and *Salmonella* at two levels of contamination: a low level at 1 CFU/test portion and a high level at approximately 10 CFU/test portion. Samples were held at 4°C for 48-72 hours before enrichment. Test method samples (450 g) were enriched with 1,800 mL of pre-warmed MP media and incubated for 10-24 hours. Unpaired reference samples (200 g and 25 g) were enriched and confirmed according to the procedures in the FDA BAM Chapter 4A for *E. coli* and Chapter 5 for *Salmonella* respectively.

Results: Test method samples were analyzed by real-time PCR. *E. coli* O157:H7 was detected in 14/20 low-inoculated samples, and *Salmonella* was detected in 18/20 low-inoculated samples. All high inoculum samples (5/5) were detected as positive. All results were identical to culture for both organisms with 100% sensitivity and 100% specificity. There was no statistical difference using the probability of detection (POD/dPOD) analysis when compared to the reference methods.

Significance: Overall, the results between the BAX® System Real-Time PCR assays and the FDA BAM reference methods were indistinguishable for detection of both pathogens in romaine lettuce.

P3-207 Validation of Cheese Powder and Blended Seasonings for the Detection of *Listeria* Using Hygiena's BAX® System

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Introduction: Cheese powders and seasonings are used across a wide range of snack foods for added flavor. The seasonings themselves do not go through any lethal processing steps and once they are applied to the snack food there is no further processing.

***Purpose*:** The purpose of this study was to validate the performance of a real-time, PCR-based method compared to the US FDA BAM reference method for the detection of *Listeria* in one cheese powder and 2 blended seasonings.

***Methods*:** Master samples of each matrix were inoculated using a lyophilized culture of *Listeria monocytogenes*. Dry dilutions of the master samples were made in uninoculated product to produce a high-level inoculum designed to achieve all positives, and one additional level to produce a low inoculum designed to achieve fractional positives. After 2 weeks of stabilization, unpaired samples for the test method (125 g) were enriched with Demi-Fraser and samples for the FDA BAM (25 g) method were enriched with BLEB and added supplements. Test samples were analyzed by real-time PCR and all samples were confirmed according to the FDA BAM Chapter 10 method.

***Results*:** Real-time PCR detected *Listeria* in 17/20 cheddar cheese low-level samples, 13/20 for seasoning 1 and 6/20 for seasoning 2. All high-level samples were positive for each matrix. Presumptive results were identical to culture with no false positives or false negatives.

Significance: The results of this study demonstrate that the BAX® System Real-Time PCR assay for Genus *Listeria* is sensitive and specific for the detection of *Listeria* species in 125 g samples of cheese powder and blended seasonings. The test method was equivalent or superior compared to the reference method.

P3-208 A *Listeria* Test Method Requiring Only a 12-Hour Enrichment in Leafy Green Matrices

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Introduction: Recent outbreaks of *Listeria monocytogenes* in leafy greens have highlighted the need for *Listeria* product testing for these matrices. Because *Listeria* is a slow-growing pathogen, commercial test methods take up to 24 hours or more, causing supply chain difficulties and issues getting product on the market with sufficient shelf life.

Purpose: To validate enrichment concentration as a method for shortening enrichment times to 12 hours for *Listeria* testing in leafy greens.

Methods: Twenty samples (125g) of romaine lettuce were inoculated with *L. monocytogenes* at 0.38 CFU/sample, a level designed to result in recovery from only a fraction of the samples. An additional five samples were inoculated at a level one log higher, and five samples were used as uninoculated controls. All samples were enriched in 250mL of pre-warmed BAC*Gro* ULTRA™ *Listeria* Broth (BULB, Gold Standard Diagnostics) and incubated at 37°C for 12 hours. Following incubation, enrichments were concentrated using the PathoTrak™ concentration system. Concentrated samples were tested for both *L. monocytogenes* and *Listeria* spp. using BACGene real time PCR kits according to manufacturer's instructions. Six additional produce matrices- broccoli, green cabbage, iceberg lettuce, kale, spinach, and Mediterranean salad mix- were also verified by inoculation of seven replicates with <10 CFU/sample and enriching and testing samples as described above.

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Results: The romaine samples inoculated at a fractional level with *L. monocytogenes* demonstrated detection by both *L. monocytogenes* and *Listeria* spp. PCR in 7/20 replicates. All five romaine samples inoculated at a higher level were positive by both PCR kits, and all negative controls showed no detection. For the remaining six matrices, all replicates showed detection of both *L. monocytogenes* and *Listeria* spp. All 144 PCR results agreed with cultural confirmation.

Significance: These data demonstrate a method to reduce *Listeria* enrichment times to 12 hours using enrichment concentration prior to PCR.

P3-209 Detection of CrAss Phage and *Cyclospora*

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Introduction: CrAssphages is a gut-associated-microbe that has been validated and used to detect fecal contamination in a variety of samples. Cyclospora is a coccidian parasite that spreads via the foodborne route. In 2023, the number of cases of cyclosporiasis in the US increased significantly compared to the three years before it. Although Cyclospora has been associated with fresh raw produce and berries, epidemiological tracebacks have not ascertained product links to infections. Although the pathogen may be present on the surface of berries, their detection has proven to be cumbersome. This project focused on whether the presence of fecal indicators may be related to the presence of parasites in berries and cilantro.

Purpose: This project explored the detection of CrAssphages as an indicator of fecal berry contamination.

Methods: Berries, and cilantro were obtained from various markets and farmers markets in the Atlanta area. 25 g of blueberries, raspberries, and cilantro and 50 g of strawberries were analyzed for the presence of CrAssphages and Cyclospora, using nPCR and qPCR in accordance with BAM protocols.

Results: part one of the study was aimed toward the standardization of sample collection, processing, and testing of detection assays to perform adequately. Part two of the study consisted of the testing of 133 samples purchased from 7 markets. Samples were examined for the presence of CrAssphages and Cyclospora. None of the samples tested positive for fecal contamination. Of the samples tested for Cyclospora, eight were determined to be presumptive positive for Cyclospora, further molecular testing is needed to confirm.

Significance: Though CrAssphages is a great fecal contamination detection assay, human associated fecal pathogens maybe present in a negative sample. Due to the presence of Cyclospora alongside a negative CrAssphage assay, it is crucial that Cyclospora detection assays be employed alongside fecal detection assays.

P3-210 Using Molecular Methods to Identify and Characterize the Establishment of *Listeria* spp. in Avocado Packing Environments

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❖ ***Developing Scientist Entrant***

Introduction: Produce industry requires environmental sampling to clear the presence of pathogens such as *Listeria* spp. This presence is commonly assessed by traditional microbiological detection methods such as culture growth and biochemistry techniques. The use of molecular methods such as PCR has been reported to provide detection of specific microbial populations.

Purpose: This study applied molecular methods in avocado packing plants to detect and identify *Listeria* spp. to trace its spreading throughout the postharvest stage.

Methods: Environmental sampling in packing plants was done in surface, floor and air applying the MLG 8.13 guidebook from USDA. Samples were enriched in Morpholinepropanesulfonic acid-buffered *Listeria* enrichment broth (MOPS-BLEB) at 35°C for 24 hours and University of Vermont Modification Medium (UVM) at 30°C for 24 hours. This was followed by DNA extractions from the enriched samples with a DNA extraction kit following the manufacturer´s instructions. Molecular confirmation of the presence of *Listeria* spp. was performed with an adapted protocol for DNA samples for BAX system Q7. Finally, DNA quantification was measured via spectrophotometer.

Results: Environmental sampling led to the obtention of 497 DNA samples. After being confirmed with PCR system, 23.1% of the samples tested positive for *Listeria* spp. The presence of *Listeria* spp. was mapped in 59% surfaces, 36% in floor and 5% air. DNA quantification resulted in concentrations >16ng/µL and ratio of absorbance 260/280 between 1.70-2.0.

Significance: Results showed the benefit of using molecular testing in packing plants as they helped identify *Listeria* spp. These findings can lead to the study of distribution and establishment of *Listeria* spp. in packing plants.

P3-211 Genome Sequence Analysis of Antibiotic Resistant *Serratia* and *Enterobacter* spp. Isolated from Imported Fresh Produce in Georgia, USA

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Introduction: The USA imports ~38% of fresh vegetables. Despite the rigorous regulations to ensure the microbiological safety of these commodities, current food safety systems do not screen for antibiotic-resistant (ABR) determinants harbored by bacteria on imported foods. Therefore, food imports can potentially spread ABR bacteria that can act as reservoirs for the dissemination of ABR determinants between countries.

Objective: Here, we performed whole genome sequencing analysis (WGS) on ABR bacteria that were isolated from fresh produce imported into the USA.

Methods: Forty imported fresh produce samples were aseptically collected from 4 major retail grocery stores in Georgia, USA. The samples (~25 g) were suspended in 100 mL of buffered peptone water in a sterile stomacher bag and gently massaged for 1 minute. An aliquot (100 µL) of the suspension was spread onto Rapid'E.coli 2 agar and incubated at 37°C for 24 hours under aerobic conditions. Morphologically distinct colonies were randomly selected, and the bacterial identity was determined using the matrix-assisted laser desorption ionization, time-of-flight mass spectrometry (MALDI-TOF MS). Phenotypic antibiotic resistance profiles were determined using the Kirby-Bauer disk diffusion assay. In-depth genomic analyses were performed using short-read Illumina sequencing, and the data were analyzed using tools available at the Center for Genomic Epidemiology.

Results: Six antibiotic-resistant isolates, including five *Serratia* spp. and one *Enterobacter* sp., were selected for genomic analyses. Using Resfinder v.4.1, it was found that the isolates harbored 1-3 acquired antibiotic resistance genes (*bla_{SRT-2}*; *tet(41)*; *aac(6')-Ic; fosA)* that encoded resistance to clinically and agriculturally important classes of antibiotics (cephalosporins, aminoglycosides, tetracycline, and fosfomycin), corroborating ABR phenotypic analyses. All the detected isolates were classified as potential human pathogens by PathogenFinder v.1.1.

Significance: This study highlighted the potential role of imported foods in the dissemination of antimicrobial resistance determinants to the USA, which requires close monitoring to avoid the rise in antibiotic-resistant infections.

Poster

P3-212 The Detection of an Extended Spectrum Beta-lactamase *Klebsiella pneumoniae* Retrieved from Fresh Parsley

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Introduction: Previously, we identified and characterized an antibiotic-resistant *Klebsiella pneumoniae* in irrigation water in Lebanon. The isolate carried *bla*SHV-187 (an extended-spectrum beta-lactamase, ESBL, gene), which was not previously reported in *K. pneumoniae* in Lebanon.

Objectives: Here, we performed in-depth analyses on a $bla_{SHV-187}$-positive *Klebsiella pneumoniae* isolated from fresh produce in Lebanon

Methods: During a nationwide study on antibiotic resistance in pre-harvest produce, a total of 60 fresh produce samples were collected from 30 different agricultural fields. In a sterile stomacher bag, the samples (25 g) were suspended in buffered peptone water (100 mL) and homogenized for 1 minute. An aliquot (100 mL) was spread on RapidE.coli2 Agar used to isolate fecal coliforms. A distinctive (mucoid green) isolate was selected, and the phenotypic resistance profile was determined using the Kirby-Bauer disk diffusion and the synergy double-disk diffusion assays. Whole Genome Sequencing (WGS) was performed using Illumina NGS platforms (NovaSeq 600), and different bioinformatics tools were used to evaluate the isolate's genomic properties. Default thresholds of percent identity and percent coverage (%) were used in ResFinder v.4.5 (90%, 60%), and PlasmidFinder v.2.1 (95%, 60%), respectively.

Results: The isolate was identified as *Klebsiella pneumoniae* (FPL9) and was detected on a fresh parsley sample. FPL9 was resistant to various antibiotic agents including colistin, a last resort antibiotic (MIC= 8 µg/mL). WGS analysis highlighted the detection of 4 acquired antibiotic resistance genes ($bla_{SHV-187}$, *oqxA10*, *oqxB25*, *fosA6*) associated with resistance against different classes of antibiotics. Moreover, the isolate belonged to sequence type (ST694) which is associated with ESBL *K. pneumoniae* in humans and animals. Further analysis showed that the isolate harbored IncFIB(K) and IncFII(pKP91) plasmids and various virulence genes (n=6). FPL9 was predicted to be a human pathogen using PathogenFinder v.1.1.

Significance: This study highlighted the contamination of fresh produce with antibiotic-resistant *K. pneumoniae* that was also detected in irrigation water, highlighting the spread of this pathogen in multiple important matrices.

P3-213 Rural Local Food Aggregation and Manufacturing Infrastructure and Technical Assistance: Lessons learned through the Share Grounds

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Introduction: Locally grown and made is trending in communities across the globe, but supporting this change is complex with many limitations to knowledge, infrastructure, supplies, and labor for supporting food production, processing, and manufacturing. The Share Grounds established in 2020 aimed to test a model for rural food aggregation and manufacturing with a series of projects, workshops, and collaborations to grow a regional food supply chain during a global pandemic.

Purpose: Establish local and regional food aggregation and manufacturing infrastructure to support an emerging Arkansas specialty crop industry and local food movement.

Methods: Three food aggregation and manufacturing facilities were established at three rural county fairgrounds. A statewide value-added outreach and technical assistance program was established to support the growth of a local and regional food supply chain in Arkansas. The team developed outreach, training and technical assistance through workshops, webinars, one-on-one client consultation, and product development in a commercial facility. The program has collaborated with the Arkansas Departments of Health, Agriculture, and Education to address regulatory, marketing, and institutional supply chain support.

Results: Over 400 clients requested technical assistance with over 1000 hours of technical support provided since June 2020. Despite the high number of requests for technical assistance and the clear need for technical support, the Share Grounds continues to struggle to meet the business needs of this emerging and fragile industry. Lesson learned about what is working and what is not will be discussed.

Significance: Locally grown aggregation and locally made food products are growing in popularity especially as states including Arkansas pass Food Freedom legislation for home-based food manufacturing. Scaling-back from a global, industrial food supply to a more regionalized food supply chain will need continued federal support as communities discover how to bridge the gap in our food system.

P3-214 Exploring Food Safety Issues and Challenges in Soilless Aquaponic Systems

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Introduction: The growing global population and increased food demand have spurred the exploration of innovative agricultural solutions. Aquaponics, a form of Controlled Environment Agriculture, emerges as a progressive, circular economy and ecologically sustainable food production system. In this approach, aquatic organisms and plants are cultivated in a soilless system, where aquaculture waste undergoes microbial changes to provide nutrients for plant growth.

Purpose: To explore key aspects of aquaponic systems, with a specific focus on addressing food safety concerns associated with this innovative agricultural approach.

Methods: A thorough scientific literature search was performed using platforms such as Google Scholar, PubMed, and Science Direct with keywords that include 'controlled environment agriculture', 'aquaponics', 'hydroponics', 'aquaculture', 'soilless agriculture', 'food safety concerns in aquaponics', 'microbiome in aquaponics', 'pathogen internalization in fresh produce'. The study involved a comprehensive review of 40 research papers related to the topic to explore the mechanisms and food safety aspects of soilless systems in comparison to conventional soil-based production system.

Results: Aquaponics was identified as a potential mitigation strategy for preventing pathogen transmission due to the minimal food safety risk associated with the absence of warm-blooded animal manure and soil. However, concerns can arise from potential internalization of human pathogens in fresh produce through plant roots immersed in water and post-harvest handling conditions and practices. Contaminated water carrying pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, and human norovirus raises food safety concerns. Further characterization of aquaponics microbial communities, especially bacterial diversity across various components, such as periphyton, plant roots, biofilter, fish feces, media grow bed zones are crucial for maintaining robust food safety measures.

Significance: Recent studies highlight aquaponics as a strategic risk management solution for food safety challenges within the broader fresh produce industry and holds promise for significantly contributing to sustainable food production with minimal environmental impact.

P3-215 Implications of Consumer Expectations and Produce Safety Regulations to the Small-Size Produce Farmer in Indiana

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❖ ***Developing Scientist Entrant***

Introduction: Many factors influence decision-making processes of growers on small produce farms. However, no prior studies have examined if consumers' expectations of food safety influence small produce farmers' attitude toward food safety compliance.

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Purpose: This study evaluated produce farmers' response to consumer expectations on produce quality and safety, as well as the impact of the Food Safety Modernization Act's Produce Safety Rule (FSMA PSR) on farmers.

Methods: At farmer conferences in Indiana between January to March 2023 and January 2024, we recruited produce farmers who qualified for exemption from the PSR. We collected qualitative data through focus groups in which we gave participants a list of key players in the food chain system and asked them to rank who they believed would be most responsible for a foodborne outbreak associated with ready-to-eat produce. We also elicited their reactions to a previous survey that identified consumers' produce safety expectations for small and large farms.

Results: We attained information saturation through five focus groups encompassing a total of 32 farmers. Participants said they perceived some benefits of being exempt from the FSMA PSR, but they were concerned about liability issues, reduced profits, and market shrinkage as well as consumers' concern regarding these exemptions. In a previous survey, consumers considered farmers the most likely members of the food chain to be responsible for a foodborne outbreak associated with ready-to-eat produce. However, in this study, farmers implicated produce packing houses as the most likely source of contamination. After learning about the prior survey, farmers became concerned about consumers' perspectives, but they believed consumers attitudes were based on insufficient knowledge. Farmers thought consumers were not aware of real-world farming practices.

Significance: The findings highlight the discrepancies between consumers' and farmers' expectations on food safety rule exemptions and provide valuable information for stakeholders and policymakers.

P3-216 Responses in Plant Secondary Metabolite Accumulations in Pre- and Post-Harvest Baby Romaine Lettuce Leaves Induced by *Escherichia coli* O157:H7

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Introduction: Little is known on how *in planta* factors influence enteric pathogens internalized into lettuce. Moreover, variability in responses may differ between live plants and harvested and processed ready-to-eat (RTE) lettuce leaves, which could have implications for food safety.

Purpose: To determine whether a) leaf secondary metabolites shift in response to internalization of *Escherichia coli* O157:H7, and b) leaf metabolite changes affect internalized pathogen populations, in live Romaine plants and store-bought RTE Romaine leaves following infiltration with an outbreak strain of *E. coli* O157:H7.

Method: A Romaine lettuce outbreak strain, *E. coli* O157:H7 2705C, was used. Leaves of live 'Carlsbad' romaine lettuce plants (4-weeks old) and store-bought RTE romaine lettuce were syringe infiltrated into the abaxial side of leaves with 100 µl of 6 log CFU/leaf of live *E. coli* 2705C (Live EC), heat-killed 2705C (Dead EC) or sterile deionized water (no pathogen control). Plants and leaves were incubated for 24 h, samples collected, and leaves lyophilized for total phenolic and flavonoid measurements. Uninoculated plants served as a reference for secondary metabolite comparisons. A second set of inoculated leaves were processed to obtain total counts for attached and internalized cells. Counts were log transformed and levels of log reduction from the inoculum were calculated. Data were analysed in JMP Pro ver. 15.2 (SAS Institute Inc., Cary, NC).

Results: Greater *E. coli* 2705C log reductions from inoculum were detected on live 'Carlsbad' plants (~1 log, $p<0.01$) than RTE leaves (0.6 log). Total phenolic and flavonoid contents increased in RTE leaves 24 h post-inoculation with live and dead EC, when compared to mock-inoculated leaves ($p<0.05$). Dead EC elicited a response in 'Carlsbad' leaves.

Significance: The lettuce outbreak strain *E. coli* O157:H7 2705C induced an increase in plant secondary metabolites in Romaine leaves.

P3-217 Transfer of *Listeria monocytogenes* and *Salmonella* from Harvest Bags to Apples

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❖ ***Developing Scientist Entrant***

Introduction: Pathogen-contaminated food contact surfaces have caused foodborne disease outbreaks linked to fresh produce.

Purpose: The objective was to quantify the transfer of *Listeria monocytogenes* and *Salmonella* from harvest bags to unwaxed apples.

Methods: Two different material-types (canvas and cordura) were cut to 5x5 cm coupons and inoculated with 5-strain cocktails of rifampicin (80ppm; R) resistant *L. monocytogenes* or *Salmonella* at ~7.3 ± 0.1 log CFU/coupon. Inoculated coupons were air-dried for 1h. Unwaxed 'Red delicious' apples from a commercial packer were weighed and placed on inoculated coupons for 5 min. Coupons and apples were sampled using the rub-shake-rub method for 60s with 0.1% peptone + 0.1% Tween80 and plated in duplicate on selective (Xylose Lysine Deoxycholate-R and Modified Oxford agar-R) and non-selective (Tryptic Soy Agar-R) media (n=30). Transfer rates were expressed as log % transfer, and linear mixed models were fitted to determine the effects of material-type and apple weight on log % transfer ($p\leq0.05$).

Results: Mean transfer rates of *L. monocytogenes* from canvas and cordura material-types was 1.17±0.29 and 1.71±0.11 log % CFU/coupon, respectively. Transfer was significantly impacted by material-type ($p<0.01$) but apple weight had no significant effect on *L. monocytogenes* transfer. Mean transfer rates of *Salmonella* from canvas and cordura material-types was 0.55±1.07 and 0.54±1.70 log % CFU/coupon, respectively. Neither material-type ($p=0.86$) nor apple weight ($p=0.58$) had a significant effect on *Salmonella* transfer. *Salmonella* transfer rates were more variable than *L. monocytogenes* transfer rates.

Significance: Pathogens can transfer to apples if harvest bags are contaminated. Transfer varied by pathogen and material-type. These findings will assist in risk management decisions by apple growers.

P3-218 Survival of *Listeria monocytogenes* and *Salmonella* on Harvest Bag Material-types

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❖ ***Developing Scientist Entrant***

Introduction: Harvest bags can be a source of microbial contamination if they are not cleaned. Assessing foodborne pathogen survival on harvest bags can aid in developing sanitation best practices (e.g., frequencies for material-types).

Purpose: Evaluating *Listeria monocytogenes* and *Salmonella* survival on two different harvest bag material-types (canvas and cordura).

Methods: Coupons (5x5cm) of each material-type were inoculated with 5-strain cocktails of rifampicin (80ppm; R) resistant *L. monocytogenes* and *Salmonella* at ~7.3±0.1 log CFU/coupon. Surfaces were air-dried for 1.5h and held at 22°C with 55% RH in a controlled growth chamber. *L. monocytogenes* and *Salmonella* were enumerated at nine time-points: 1.5, 4, and 8h, and 1, 2, 3, 7, 14, and 21d post-inoculation. Coupons were rubbed and shaken for 60s with 0.1% peptone and plated in duplicate on selective (Modified Oxford Agar-R and Xylose Lysine Deoxycholate Agar-R) and non-selective (Tryptic Soy Agar-R) media in triplicate experiments with triplicate replicates (n=9). Significant differences ($p\leq0.05$) were evaluated by Tukey's HSD test in R-Studio (version 4.3.1). Segmented linear models were also fitted to determine the relationship between time and pathogen recovery in log CFU/coupon.

Results: *L. monocytogenes* decreased at 21 d by 3.2 ± 0.8 and 3.1 ± 0.9 log CFU/coupon on canvas and cordura material types, respectively. *Salmonella* decreased at 21 d by 1.9 ± 0.5 and 2.2 ± 0.4 log CFU/coupon on canvas and cordura material types, respectively. With both pathogens, there was variation in reduction based on material type. Segmented models showed biphasic die-off rates with a breakpoint at 0.37 (R^2=0.82) for *Listeria monocytogenes* and triphasic die-off rates with breakpoints at 1.13 and 9.04 d for *Salmonella* (R^2= 0.92).

Significance: The survival of these pathogens was impacted by material-type, and time-point with *L. monocytogenes* exhibiting a greater die-off than *Salmonella* over time. Frequent cleaning (e.g., daily) of these bags is recommended.

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P3-219 Evaluation of Sodium Hypochlorite and Peracetic Acid Efficacy in Prevention of Cross Contamination during Baby Spinach Washing Process

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❖ ***Developing Scientist Entrant***

Introduction: Addition of antimicrobials to water during fresh produce washing can significantly reduce microbial food safety risk. However, the organic compounds released from produce may interfere with the efficacy of antimicrobials. Thus, there is a need to validate the efficacy of these antimicrobials in wash water with a range of dissolved organic compounds.

Purpose: To evaluate the antimicrobial performance of sodium hypochlorite (NaOCl) and peracetic acid (PAA) during a lab-scale flume washing process using baby spinach.

Method: Unwashed baby spinach leaves were collected from a local farm in Maryland, stored at 4°C and used within two days of harvesting. Twenty ml of a 24-hour *E. coli* TVS353 culture was inoculated dropwise onto leaf surfaces (final concentration of 8 log CFU/leaf) and air-dried at room temperature in a biosafety cabinet. Wash water at the targeted chemical oxygen demand (COD) levels (300 or 2500 ppm) was prepared using ground spinach and soil suspensions. Sanitizer solutions were added into the wash water to achieve the targeted concentrations of 20 and 80 ppm. For each replicate (n=6), one inoculated spinach leaf was washed with nine uninoculated leaves in 500 ml wash water on an orbital shaking at 100 rpm for 1 min. After neutralization, leaves and wash water were taken for bacterial enumeration using a modified most probably number (MPN) method.

Results: The bacterial inactivation efficiency was sanitizer dose-dependent for PAA but not NaOCl. At 300 ppm COD, 40 ppm PAA or 80 ppm NaOCl, cross-contamination was effectively prevented during the washing process (>5 log CFU/ml inactivation). At 2,500 ppm COD level, 80 ppm PAA or 80 ppm NaOCl were required to achieve the same effect.

Significance: The results illustrated adequate sanitizing efficacy of NaOCl and PAA during the baby spinach washing process and PAA concentration could be reduced at lower COD levels.

P3-220 At-Harvest Treatment of Romaine Lettuce with Lytic Bacteriophage is not an Economically Practical Mitigation for Potential REPEXH Contamination

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Introduction: The leafy greens industry speculated that lytic bacteriophage would provide a tool for recuring food safety risks. Reticence to apply phage postharvest has persisted. At-harvest applications were hoped to provide mitigation.

Purpose: A coalition of California leafy greens handlers requested an independent assessment of the efficacy of a commercialized anti-*E. coli* O157:H7 bacteriophage (O157phage), EcoShield PX™, at harvest.

Methods: Laboratory tests were conducted to determine quantitative and qualitative log-reductions of commensal *E. coli*, an attenuated *E. coli* O157:H7, and four virulent *E. coli* O157:H7 isolates on field-harvested Romaine lettuce. Formulations were provided by Intralytix, Inc. Comparative efficacy included pre and post-phage treatment of ten independent replicates of 25g tissue at log 3 and 6 CFU bacteria/sample and held at 4C for 48h, preceded or followed by O157phage at log 6,7,8, and 9 PFU/sample, hypochlorite at 4, 10, and 15 mg/L at pH 7.0 (applied pre or post treatment like a typical chlorinated "box spray" mounted on harvesters), varying hold temperatures, and 4, 16, 24 or 48 h hold times prior to plating or enrichment-assisted qPCR below the limit of detection (LOD 5 CFU/25g). Residual lytic O157phage activity was assessed by phosphate buffered saline eluates from EcO157 inoculated and O157phage treated but non-inoculated leaves by soft-agar plaque assays.

Results: Recovery of applied bacteria was not significantly different from controls at economically acceptable levels of O157phage treatment. Pre-contamination applications of O157phage had no discernable effect on viable EcO157 recovery. Log reduction of EcO157 isolates was not significantly different from pre-sprays of hypochlorite and not as effective as chlorination when applied alone at titers less than log 9 PFU/ml. Residual O157phage plaques from lettuce leaf washate were ≥ log 5.5 PFU/sample.

Significance: Results do not support a cost-effective at-harvest mitigation of REPEXH by O157phage on Romaine lettuce and continued advocacy for funding by academia is highly questionable.

P3-221 Antimicrobial Efficacy of TiO2 against *Listeria*, *Salmonella,* and *E. coli* in Microgreen Systems

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❖ ***Developing Scientist Entrant***

Introduction: Microgreens are superfoods that have grown in popularity. Recent outbreaks have highlighted several food quality and safety risks: microgreens are harvested shortly after germination in a warm and moist environment. Titanium dioxide (TiO2) has been used to inactivate microorganisms through photocatalytic generation of reactive oxygen species. This technology is stable, non-toxic, inexpensive, and operates under ambient conditions.

Purpose: This study aims to evaluate the efficacy of TiO_2 as a potential antimicrobial for microgreen systems.

Methods: TiO_2 antimicrobial activity was tested against *Listeria, Salmonella, and E. coli* at 1, 5, and 10 % w/v for up to 24 hours *in vitro* conditions. At appropriate time intervals, bacterial population was enumerated. The best conditions for microbial reduction were used in the microgreen growing systems where arugula seeds were experimentally inoculated. Germination and growth were monitored for 10 days, and bacterial growth, color, antioxidants, phenolic concentrations, and visual appearance were evaluated. Two growing systems were run in parallel: a control tower where no TiO_2 was applied, and a treatment tower where TiO_2 was integrated by a water tank reservoir system. Experiments were conducted in triplicate and a significant *P* value was set at 0.05.

Results: Pathogens were detected in the edible part of the microgreens at harvest. A significantly lower ($p<0.05$) microbial concentration was detected in samples that were grown from contaminated mats (6.7 log CFU/g), as compared to when seeds were contaminated (8 CFU/g). These results suggest that both contamination routes pose a relative risk, as pathogens could survive. Nevertheless, when the TiO_2 treatment tank was used, *Listeria* population was significantly reduced by the end of the experiment. No significant difference ($p>0.05$) in appearance, color, or other physical-chemical parameters was observed.

Significance: This study provides insight into the potential efficacy of TiO2 to reduce the risk of foodborne pathogen growth in microgreen systems.

P3-222 Burden of Foodborne Illness Associated with *Salmonella*-Contaminated Onions

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Introduction: Despite the increased size and frequency of outbreaks involving the contamination of onions with nontyphoidal *Salmonella* over recent years little has been done to evaluate the illness and economic burdens associated with these outbreaks.

Purpose: In this study we assess annual rates of illness and economic costs associated with *Salmonella*-contaminated onions in the United States.

Methods: To assess illness rates, we use outbreak data from the National Outbreak Reporting System (2004-2021), supplemented with data from CDC's listing of multistate foodborne outbreaks (2022-2023). Mean outbreak estimates for illnesses associated with nontyphoidal *Salmonella* and onions are converted into nationwide burden estimates using published multipliers for underdiagnosis and outbreak to case-confirmed illness. Results for alternative

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multiplier values are examined in a sensitivity analysis. Economic cost estimates are derived by combining illnesses with published cost of illness estimates (updated to reflect changes in costs). Uncertainty in model parameters is preserved using Monte Carlo analysis (10,000 iterations) in @Risk 7.5.

Results: Between 2004 and 2023 a total of 9 onion-related *Salmonella* outbreaks were associated with an annual average of 130 illnesses. Accounting for outbreak to case confirmed and underreporting factors yields an estimated annual mean of 60,543 (90% CI: 46,928-83,133) illnesses. Given an average cost per case of $7,027 (90% CI: 2,109-17,672), the mean annual expected cost of illness from onions contaminated with *Salmonella* is $414 million (90% CI: $136-$1,016 million).

Significance: Understanding the substantial human illness and economic burdens associated with *Salmonella*-contaminated onions gives policymakers, researchers, and industry stakeholders an important tool that can be used to evaluate and prioritize food safety efforts.

P3-223 A Brush With *Listeria*: Evaluation of Peach Packing House Hygiene

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❖ ***Developing Scientist Entrant***

Background: Produce-associated outbreaks and recalls have been traced back to packinghouse contamination. Packinghouse equipment can accrue microbial load from produce surfaces. Areas such as floors, drains, and brushes can serve as harborage sites for food pathogens.

Purpose: In this study, Heterotrophic plate count (HPC) and *Listeria* spp. presence was evaluated for environmental samples (Food contact surfaces (8) and non-food contact surfaces (4)) collected from peach packing houses throughout the production season.

Methods: HPC was obtained by plating on Tryptic Soy Agar (TSA) after diluting the swab samples with 1x PBS. The presence/ absence of *Listeria* spp. was identified by enriching the swab samples with BLEB (Buffered Listeria Enrichment Broth) followed by addition of antibiotics and streak plating on RAPID'L. mono agar after 24 – 48h incubation at 30 ºC.

Results: A significant difference in HPC was observed between two packing facilities (P1 HPC: 4.28±1.67; P2 HPC: 3.38±1.42) ($p \leq 0.05$). The mean HPC during end (4.24±1.63 log CFU/cm^2) and mid (4.12±1.68 log CFU/cm^2) of packing seasons was significantly higher ($p \leq 0.05$) than the early-packing season (3.06±1.22 log CFU/cm^2). Waxer brushes in zone 1 of both PH1 and PH2 were found to be heavily contaminated (HPC: 5.49±0.89 log CFU/cm^2) ($p \leq 0.05$). *Listeria* spp. was identified during pre-sanitation mid-season sampling in both PH1 and PH2. *L. monocytogenes* was identified in one of the swab samples from the cold storage drains of PH1. *L. welshimeri* and *L. innocua* were identified on the same day from waxer brushes and cold storage drains (PH1) respectively. *L. welshimeri* was also found in the waxer brushes swab samples from PH2.

Significance: These results suggest that there was an increase in microbial load throughout the production season and waxer brushes serve as good harborage sites for pathogens like *L. monocytogenes*.

P3-224 Genotypic and Phenotypic Characterization of Salmonella enterica Isolates from Wastewater

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella enterica* is a foodborne pathogen that contributes to the global pandemic of antibiotic resistance, which results in the emergence of pathogens with resistance to clinically important antibiotics.

Purpose: Genotypic and phenotypic methods were used to conduct an analysis of antimicrobial resistance (AMR) in environmental *Salmonella* isolates.

Methods: Wastewater samples were collected from the City of Guelph (Ontario, Canada) Wastewater Treatment Facility on four occasions between April and July 2023. *Salmonella* were isolated from wastewater influent by immunomagnetic separation, and then inoculated into Rappaport-Vassiliadis soya peptone broth and Brilliant Green 2% broth, and incubated at 42.5°C overnight. Enrichments were streaked onto selective agars (Bismuth Sulfite agar, Xylose Lysine Deoxycholate agar, Brilliance *Salmonella* agar) and incubated at 42.5°C overnight. Suspect colonies were confirmed as *Salmonella* using biochemical analysis. Antibiotic susceptibility testing (AST) using the Kirby-Bauer Disk Diffusion method was performed. The *Salmonella* isolates were subjected to short-read whole genome sequencing (WGS) using the Illumina MiniSeq platform, followed by assembly and annotation using the Bacterial and Viral Bioinformatics Resource Center. Serovar analysis was completed using the *Salmonella* In Silico Typing Resource (SISTR) pipeline and AMR gene analysis using the Comprehensive Antibiotic Resistance Database (CARD). Genomic epidemiology of the sequences was conducted by comparing to Canadian outbreak associated *Salmonella* WGS sequences.

Results: The *Salmonella enterica* isolates belonged to four serovars (Hartford, Typhimurium, Thompson and Stanley). *In silico* analysis of AMR using CARD identified the presence of a number of resistance genes including fluoroquinolones and aminoglycosides. Phenotypic AST results confirmed that all isolates were of intermediate resistance to streptomycin and 5/11 were resistant to ciprofloxacin. No strains linked to outbreaks in Canada. Paired student T-test analysis revealed significance between the two tested phenotypic AST methods ($p<0.05$).

Significance: Surveillance of antibiotic resistant *Salmonella* in wastewater influent can provide insight into the emergence of new AMR patterns, and provide important information regarding clinical treatment leading to improved patient outcomes.

P3-225 Five Years of *Listeria monocytogenes* Surveillance in Chilean Watersheds

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that can enter food by several routes, including contaminated water. In addition, this pathogen can form biofilm under different stress conditions, which allows it to survive in the food environment.

Purpose: To analyze the prevalence of *L. monocytogenes* for 5 years (2019-2023) in two Chilean watersheds, Mapocho (MAP) and Maipo (MAI), and to evaluate the genomic diversity and biofilm-forming capacity of isolates.

Methods: We monitored 30 sampling sites over five years (MAI, 780 samples) and (MAP, 720 samples), including different water types (river, irrigation canal, creek, and pond). *L. monocytogenes* was identified by microbiological culture and confirmed by PCR. In addition, 129 isolates were sequenced, and 15 strains were tested for biofilm formation at 37 and 8°C using the crystal violet method.

Results: A high prevalence of *L. monocytogenes* was found in 2019 (MAP: 22.9%; MAI: 26.7%) and in 2023 (MAP: 28.3%; MAI: 47.8%). From 2020 to 2022, the average *Lm* detection was lower (MAP:15.9%; MAI:11.3%). In all samples collected from irrigation canals (MAP: 18; MAI: 17), *L. monocytogenes* was detected at least twice during the entire sampling period, with frequencies ranging from 7% to 54%. Genomic analysis showed that the *L. monocytogenes* IVb serogroup was predominant in both watersheds (MAP 54%, 36/66; MAI 59,6%, 37/62) while the IIa and IIb serogroups showed lower prevalence. All *L. monocytogenes* strains were able to form biofilm at 37°C and 8°C. The ability to form biofilm was not related to the serogroup of the isolates.

Significance: This study highlights the persistent prevalence of *L. monocytogenes* in Chilean watersheds, indicating an ongoing risk of food contamination. The genomic diversity and biofilm formation capacity highlight the adaptability of *L. monocytogenes* in aquatic environments and emphasize the need

for effective control strategies.

P3-226 A Machine Learning Model to Predict the Presence of Salmonella in Agricultural Water Resources

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❖ *Developing Scientist Entrant*

Introduction: Surface water is crucial for agricultural irrigation, and its contamination by pathogens, such as *Salmonella*, poses risks to public health. In this study, we investigated the presence of *Salmonella* in surface waters and its association with environmental and anthropogenic factors.

Purpose: Our goal is to predict Salmonella presence or absence in water samples for agricultural use in the VI and Metropolitan Region of Chile through Machine Learning techniques

Methods: Water samples were collected from April 2019 to March 2023 at different sites in the O'Higgins and MR region. *Salmonella* detection followed the FDA-BAM protocol, confirmed using PCR with the invA gene. Environmental and anthropogenic factors were recorded during sample collection. A Random Forest statistical analysis was conducted to identify factors associated with *Salmonella* presence.

Results: During this period, *Salmonella* was detected in 32.4% (719/2219) of the analyzed samples, out of a total of 2219 processed samples. Random Forest analysis highlighted that water's physical parameters significantly influenced *Salmonella* detection. Key factors included pH (Importance Score (IS) = 0.1317), conductivity (IS = 0.1218), salinity (IS = 0.1207), total dissolved solids (IS = 0.1168), and water temperature (IS = 0.1167). Conversely, the presence of animals, their feces, human settlements (IS = 0.0082), and human feces (IS = 0.0087) showed weaker associations with *Salmonella* detection. It is important to note that the sum of all IS values equals 1.0, reflecting the relative weights of these factors in the model. The model demonstrated substantial capability in predicting *Salmonella* absence, with a precision of 67% and an F1-score of 0.61. However, the model exhibited limitations in predicting *Salmonella* presence, with a precision of 41% and an F1-score of 0.46, indicating areas that require further refinement.

Significance: Based on the results obtained, our approach allows us to optimize the detection of *Salmonella* in irrigation waters by prioritizing resources in sites with a higher probability of the pathogen's presence, particularly those exhibiting the most influential risk factors identified, such as pH levels in water. This not only supports efforts in food safety and public health but also drives significant improvements in food protection and public health, especially in regions with limited resources and developing countries.

P3-227 Industry Reaction to Proposed Subpart E and Perceived Barriers to Treating Production Water

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Introduction: The FDA proposed new Produce Safety Rule requirements for production water (Subpart E) in December 2021. The focus of Subpart E shifted from testing to an assessment-based decision-making model. It is unclear if growers are utilizing water treatment as a corrective or mitigation measure or perceive the proposed requirements as easier to implement.

Purpose: To describe fresh produce grower responses to the proposed rule for production water management, query if growers are currently treating production water and why, and identify the most significant barriers growers face when treating production water.

Methods: An anonymous survey was distributed online via Qualtrics™ to the produce industry from March to August 2023. Descriptive statistics were used to determine frequencies and distribution of responses. Ranking of barriers to effective production water treatment were assessed by inversely weighting, after which a score was calculated for each priority by summing weighted rankings across responses. Responses were analyzed using descriptive statistics in RStudio version 4.2.3.

Results: Forty-six respondents completed the survey (n=46), identifying primarily as growers (36/46; 78.3%). Significant differences were observed between growers' and other roles' responses ($p<0.05$); thus, responses were evaluated separately. Approximately half of respondents reported having read the proposed rule (15/32) and poor understanding of proposed requirements (20/40). Forty-three percent of growers reported that the proposed rule was more difficult to implement than the previous version (13/30) but that training would help compliance (27/36). Less than 30% of growers reported treating their production water (9/36). Of those, most (6/8; 75.0%) are doing so for general risk management, followed equally by buyer and regulatory requirements (5/8; 62.5%). The largest reported barrier that growers identified when treating water was the impact of treatment on soil health (weighted ranking=19).

Significance: Most growers that responded were not treating production water; however, the final requirements for Subpart E emphasize water treatment as a corrective or mitigation measure, which growers will have to address.

P3-228 Assessing the Presence of Foodborne Pathogens in Agricultural Water from Produce Farms in Alabama

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❖ *Developing Scientist Entrant*

Introduction: Agricultural water is a well-known source of microbial contamination that significantly impacts the fresh produce industry and public health by causing numerous outbreaks and recalls annually. Alabama has over 2,000 produce farms, but most are exempt from the Produce Safety Rule, which can represent a risk to public health.

Purpose: This research aimed to investigate the prevalence of foodborne pathogens and indicator organisms from surface water.

Methods: Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella,* and *Escherichia coli* were measured from produce farms in Alabama. Bi-monthly water samples (500 mL) from seven irrigation ponds were collected from January 2022 to December 2022 (N = 174). Generic *E. coli* was enumerated using the IDEXX Colilert with Quanti-Tray/2000 method. Samples were pre-enriched and cultured for *Salmonella* isolation using a modified FDA BAM method and isolates were further confirmed by PCR analysis (*invA*). *Salmonella*-positive samples were further submitted for serotyping. STEC genes (*hly, fliC, eaeA, rfbE, stx*-I, *stx*-II) were surveyed by PCR from sample enrichment.

Results: Overall, populations of generic *E. coli* ranged from 1 to 4 log MPN/100 mL. *Salmonella* was detected in 12/174 (6.89%) water samples, from which 3 serotypes were identified: Hartford, Inverness, and Newport. The STEC genes were detected in 157/174 (90.20%) samples, with prevalence varying amongst genes (*hly* 137/174 (78.735%), *fliC* 91/174 (52.29%), *eaeA* 47/174 (27.01%), *stx*-II 34/174 (19.54%), *rfbE* 28/174 (16.09%), and *stx*-I 14/174 (8.04%)). *Salmonella* and STEC isolates were found throughout the whole sampling period.

Poster

Significance: Assessing the prevalence of the aforementioned organisms in produce farms is important to better understand the potential risk that farms exempt from the PSR represent to the safety of the produce grown in Alabama.

P3-229 Evaluation of Dead-End Ultrafiltration (DEUF) and Modified Moore Swab (MMS) Methods for the Recovery of *Campylobacter* Species from Agricultural Water

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Introduction: *Campylobacter* spp. is the leading cause of gastroenteritis worldwide. Campylobacteriosis is usually associated with the consumption of raw milk, undercooked poultry, contaminated drinking water or seafood. However, *Campylobacter* spp. have been isolated from soil, surface water, and groundwater, which suggests that they could be transferred to food crops. Therefore, it is critical to a develop sensitive and practical method to recover *Campylobacter* spp. from agricultural water.

Purpose: To compare the recovery of *Campylobacter* spp. from spiked agricultural water using two filtration methods, DEUF and MMS.

Methods: Agricultural water (10 L) was spiked with known concentrations (<1-1000 colony-forming units [CFU]) of *Campylobacter* spp. (*C. jejuni*, *C. coli*, or *C. lari*) and filtered through either a dead-end ultrafilter or MMS. Each MMS was directly enriched in Bolton or Preston broth under microaerobic conditions at 37 and 42°C for 48 hours. Colonies were recovered on modified charcoal cefoperazone deoxycholate (mCCDA) or chromogenic agar plates. The ultrafilters were backflushed with the elution buffer; eluates were centrifuged at 8000 rpm for 30 minutes and the recovered pellets were enriched as described above. In addition to PCR confirmation, colonies were examined using a phase contrast microscope, for motility and *Campylobacter*-like morphology.

Results: Our results showed that the limit of detection for *Campylobacter* spp. was below 10 CFU/10 L for DEUF, and above 50 CFU/10 L for MMS. The results were similar when either Bolton or Preston broth was used for the primary enrichment.

Significance: The MMS and DEUF methods have been used in the field for filtration of large volumes of water. Our results show that the DEUF method offers great sensitivity for the recovery of low numbers of *Campylobacter* spp. from large volumes of water. Once validated, FDA will have a sensitive method for detection of *Campylobacter* spp. in agricultural water.

P3-230 Prevalence of Genes Associated With *Shiga* Toxigenic *Escherichia coli* in Agricultural Ponds: a Longitudinal Study

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❖ ***Developing Scientist Entrant***

Introduction: Proposed changes to the produce safety rule require hazard analysis of each agricultural water source and the accuracy of such analysis may be improved by focusing data collection efforts on factors affect pathogen presence.

Purpose: Develop an understanding of the physical and chemical factors that are associated with the occurrence of *Shiga* toxin-producing *Escherichia coli* (STEC) in agricultural ponds.

Methods: Water samples (0.5L) were collected with physicochemical water quality data across sampling grids spanning the perimeter, surface and subsurface of three ponds and were enumerated for *E. coli* using Quanti-tray 2000 MPN (IDEXX). A multiplex real-time PCR was used to detect the presence of three genes associated with STEC (*eae, stx1, stx2*) in presumptive *E. coli* colonies isolated from MPN trays after enumeration. Fluorescent wells (*E. coli*-positive) were isolated on MacConkey agar and CHROMagar *E. coli*, while non-fluorescent wells were isolated on CHROMagar O157. A random forest (RF) model (package ranger) and binomial logistic regression (logit) were created to determine factors driving presence of STEC genes.

Results: 190 isolates (144 fluorescent and 46 non-fluorescent) were analyzed, 6 of which were positive for at least one STEC target. Pond 1 (n=73) had one isolate positive for *eae* and another for *stx2*. Pond 2 (n=74) had a *stx1* and a *stx2* positive isolate. Pond 3 (n=43) had two isolates positive for *eae*, one from a water sample negative for generic *E. coli,* but presumptive positive for *E. coli* O157:H7. RF analysis showed sample area (surface, subsurface, perimeter) as the most important splitting factor. The logit test for STEC target presence by sampling area for the three ponds showed detection was significantly (p<0.05) more likely at the perimeter and surface.

Significance: Sampling location may significantly affect the probability of detecting STEC in irrigation ponds. Water quality sampling for compliance with food safety standards may need to account for these differences.

P3-231 Evaluation of the Microbial Populations and Physicochemical Profiles of Harvested Rainwater and Municipal Water Used for Crop Irrigation

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Introduction: In urban agriculture, harvested rainwater practices are growing due to limited resources and reliable access, though the impacts on potential contamination of crops through irrigation with harvested and stored rainwater have not been fully explored.

Purpose: This study was designed to evaluate harvested rainwater (HR) and municipal water (MW) irrigation sources through common water quality indicators. The physicochemical profiles, E. coli and total coliform (TC) levels were monitored to provide information about potential pathogen presence and food safety risks.

Methods: HR and MW samples (n=8) were collected for 4 weeks prior to harvest of spring mix lettuce. Rainwater was captured from rooftop runoff and stored in a 500-gallon barrel until irrigation and sample collection. *E. coli* and TC levels were determined using IDEXX Colilert assay. Total dissolved solids (TDS), heavy metal, and nutrient testing were performed. Statistical analyses were performed using Student's t-test.

Results: *E. coli* and TC levels were below the detection limit, 1 MPN/100 mL, for all MW samples. In HR, average TC levels decreased from 15,163 to 765 MPN/100 mL over the 4-week period and average E. coli levels decreased from 164 to 4.1 MPN/100 mL in the same timeframe. Temporal changes could be due to bacterial die-off and dilution with newly harvested rainwater during the sampling period. Average TDS levels (ppm) for HR and MW were significantly different (p<0.05) at 435±128 and 169±30, respectively. Average calcium, magnesium, and sodium levels (mg/L) were significantly lower (p<0.05) in HR (3.82±0.61, 1.07±0.19, and 0.76±0.07, respectively) than MW (52.11±3.83, 12.53±1.16, and 23.89±2.23, respectively). Alternatively, average ammonium and nitrate levels (mg/L) were significantly higher (p<0.05) in HR (121.4±30.3 and 123.0±37.2, respectively) than MW (10.8±9.5 and 8.7±7.9, respectively).

Significance: This research characterizes the microbial and physicochemical quality of harvested and stored rainwater and the potential food safety risks associated with its use as an irrigation source.

Poster

P3-232 Inactivation of *Listeria monocytogenes* by Chlorine Dioxide in Agricultural Water is pH Dependent

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Introduction: Disinfectants are used in agricultural water to prevent bacterial contamination. However, agricultural water quality can vary in pH and organic material. The effectiveness of chlorine dioxide (ClO2), against bacterial pathogens, like *Listeria monocytogenes,* may depend on water quality.

Purpose: Therefore, the objective of this study is to understand how factors like pH and organic material affects the inactivation of *L. mono* by ClO_2

Methods: The minimum inhibitory concentration (MIC) of ClO_2 to produce a 3-log reduction of *L. monocytogenes* was evaluated in double-deionized water (control) and two standardized water samples prepared following protocol set forth by the Environmental Protection Agency (EPA). The EPA standardized water was adjusted to pH 6.5 and 8.4. A stock solution of ClO_2 was diluted with the water samples and inoculated with a cocktail of *L. monocytogenes*. The pathogens were exposed to the ClO_2 for 5 minutes and sampled for viable cells using Palcam agar. The plates were incubated for 48 hours prior to enumeration.

Results: A statistically significant difference (N=3; $p<0.05$) in the MIC was observed between sterile double-deionized water and pH 6.5 EPA water but not between sterile double-deionized water and pH 8.4 EPA water. The MIC of ClO_2 for a 3-log reduction of *L. monocytogenes* was 10 mg/L for sterile double-deionized water and pH 8.4 EPA water. In comparison, pH 6.5 EPA water had a MIC of 2.5 mg/L.

Significance: This study suggests that pH has an impact on the survival of *L. monocytogenes* when treated with ClO2. This information is useful when determining sanitizer application protocols when targeting *L. monocytogenes* in agricultural water.

P3-233 Reactivity of pH Adjusting Agents with Chlorine and Formation of Trichloromethane in Chlorinated Wash Water

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Introduction: Chlorine is commonly used by the fresh produce industry to sanitize wash water and minimize pathogen cross-contamination. To maximize the antimicrobial efficacy of chlorine, the pH of chlorinated water is often reduced to values of pH 6-7 with citric acid. Earlier studies have demonstrated that citric acid reacts with chlorine forming high amounts of trichloromethane, a major chlorine byproduct in water, and a probable human carcinogen. However, it is unclear if other organic or inorganic acidifiers can replace citric acid to minimize the formation of trichloromethane.

Purpose: The objective of the present study was to determine the reactivity of organic and inorganic pH adjusting agents with chlorine and their potential generation of trichloromethane.

Methods: Chlorine (~100 ppm) was mixed with 10 mM of each of twelve organic acids (including citric acid), phosphoric acid and sodium acid sulfate to affect a pH level of 6.5. Free chlorine levels and trichloromethane concentrations were measured over 3 h at 3°C and 22°C.

Results: Ascorbic acid, dehydroascorbic acid, citric acid, and malic acid rapidly depleted levels of free chlorine at both 22°C and 3°C, while tartaric acid and lactic acid decreased chlorine levels more slowly during 3 h of incubation at 22°C. Using citric acid and malic acid as pH adjusting agents led to the formation of significantly ($p<0.05$) higher concentrations of trichloromethane than other acids. Even though ascorbic acid and dehydroascorbic acid rapidly depleted chlorine, low or no significant concentrations of trichloromethane were formed. Phosphoric acid and sodium acid sulfate did not lead to the depletion of free chlorine or formation of trichloromethane.

Significance: Our results demonstrated that sodium acid sulfate, phosphoric acid and some organic acids can be used to replace citric acid as pH adjusting agents in chlorinated water.

P3-234 Evaluating the Recovery of Pan-susceptible and Antibiotic-resistant *Escherichia coli* in Synthetic Test Agricultural Water Using Membrane Filtration and Colilert Methods

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Introduction: Membrane filtration and Colilert/Quantitray assays are commonly used to quantify *Escherichia coli* levels in agricultural water. These methods have not been assayed in "test agricultural water" (TAW), a formulation proposed by the Environmental Protection Agency (EPA) for assessing effectiveness of chemical sanitizer treatments on pathogens in water. The recovery efficiency of these methods for pan-susceptible and antibiotic-resistant *E. coli* in TAW should be evaluated.

Purpose: To evaluate the recovery of *E. coli* from TAW at different turbidity levels using membrane filtration and Colilert methods.

Methods: TAW was formulated using EPA protocol with a pH of 6.5 at two turbidity levels: no added turbidity (NT, < 5 NTU) and 50 NTU, achieved by adding PTI Arizona test dust. Pan-susceptible *E. coli* ARS C101 and cefotaxime-resistant *E. coli* ARS C301 were added separately to TAW (200 CFU/100 ml). Inoculated TAW was either filtered using a 0.45 µm cellulose ester membrane filter and placed onto Tryptone Bile X-Glucuronide (TBX) agar, CHROMagar-ECC (ECC), or tested by IDEXX Quanti-Tray/2000 Colilert. For recovery of *E. coli* C301, media were supplemented with 4 µg/ml cefotaxime. A two-way ANOVA was used to test differences ($p<0.05$) between recovery percentages from different media/methods. Experiments were performed in triplicate.

Results: Recovery percentages of both *E. coli* C101 and C301 at both turbidity levels were similar on TBX and ECC, between 88.8% and 121.7%. For Colilert assays, the recovery percentage of *E. coli* C101 in NT and 50 NTU was 118.3±6.7% and 94.6±18.6%, while *E. coli* C301 in NT and 50 NTU was 127.0±10.5% and 93.4±4.4%, respectively. There was no significant difference in recovery percentages between C101 and C301 strains at either turbidity level on TBX, ECC or by the Colilert method ($p>0.05$).

Significance: Membrane filtration and Colilert method recovered equivalent percentages of pan-susceptible and antibiotic-resistant *E. coli*, regardless of turbidity levels in TAW.

P3-235 Antimicrobial Resistance Genes in *Salmonella* from Latin America Surface Waters

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Introduction: Surface water is used for irrigation in food production worldwide. These waters could be contaminated with diverse antimicrobial-resistant bacteria and genes, which have been recognized as an increasing threat to public health.

Purpose: To determine the diversity of genes and mutations associated with antimicrobial resistance (AMR determinants) in *Salmonella* collected from surface waters from Brazil, Chile, and Mexico between 2019 to 2022.

Methods: From 2019 to 2022, 1,911 *Salmonella* isolates were recovered from surface waters in Brazil (n=541), Chile (n=685), and Mexico (n=685). They were sequenced, and the presence of AMR determinants was detected using NCBI/AMRFinderPlus. AMR resistance to antimicrobial families was predicted using the Bacterial AMR Reference Gene Database (NCBI). Statistical analysis (Kruskal-Wallis) was conducted in Past4.03.

Results: The collection included 78 AMR determinants linked to 13 AMR families. One-third (33.9%;647/1911) of the genomes studied contained from 1

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to 17 AMR determinants. The most frequently found were the quinolone resistance gene *qnrB19* (14.3%), followed by *tet(A)* (tetracyclines; 13.65%) and *floR* (phenicols; 10.51%). The most frequently found mutations were to gene *gyrA* (n=4; 110/1,911 genomes). The number of AMR determinants per genome varied significantly between countries, with averages of 0.41 for Brazil, 1.22 for Chile, and 2.24 for Mexico. Most isolates were resistant to quinolones (22.7%), followed by tetracycline (15.7%) and Aminoglycosides (14.6%). In general, Mexican isolates presented the greatest proportion of resistant isolates to all AMR families. Finally, 16% of genomes were expected to be Multidrug-resistant, including 5.5% from Brazil, 13.9% from Chile, and 26.4% from Mexico.

Significance: Surface waters contaminated with multidrug-resistant *Salmonella* could potentially reach consumers through vegetable-origin foods and cause difficult-to-treat infections. Monitoring their presence in surface waters could aid decision-makers in developing mitigation solutions for this hazard to global health.

P3-236 Antimicrobial Resistance in Surface Waters of Florida and Arkansas across Hot Spots of Urban, Agricultural, and Natural Environments

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Introduction: Antimicrobial Resistance (AMR) has been closely monitored in select pathogens of humans and animals for many years, however significant data gaps exist regarding the prevalence of AMR across the environment. The National Antimicrobial Resistance Monitoring System (NARMS) has identified surface waters as an integral matrix that will provide a wealth of highly relevant data regarding baseline prevalence of AMR across the United States. A strategy has been implemented to sample statistically significant "hot spots" of surface water from urban, agricultural, and natural sites to better understand AMR baselines in the environment and the selective pressures that may impact their prevalence.

Purpose: To describe AMR in surface water from agricultural, urban, and natural environments in Arkansas and Florida to generate a baseline understanding of AMR prevalence as well as associated microbiota. To better understand how selective pressures associated with anthropogenic and natural environments impact the prevalence of AMR.

Methods: Hotspot analysis was conducted using land cover data to identify sampling sites. Surface water was collected from sites across Arkansas (n=15) and Florida (n=9). Metagenomic and quasimetagenoimc sequence data (AR; n=30, FL; n=27) was described using the AMRplusplus, AMRfinderplus and CARD pipelines.

Results: Critically important Antimicrobial Resistance Genes (ARGs) were identified in Arkansas (n=31) and Florida (n=26) quasimetagenomic data. ARGs belonged to Polymyxin (AR; n=1, FL; n=1), Macrolide (AR; n=5, FL; n=5), β-lactam (AR; n=13, FL; n=12), and Fluoroquinolone (AR; n=12, FL; n=8) classes. Dominant bacterial taxa included: *Polynucleobacter, Synechococcus*, *Aeromonas* spp., and *Vibrio* spp. Using traditional culture-based techniques, *Salmonella enterica* was recovered from 60% (9/15) of Arkansas sampling sites and 77% (7/9) of Florida sampling sites.

Significance: The methods described here provide a framework for supporting One Health AMR monitoring of surface waters to describe baseline AMR prevalence, associated bacterial species as well as selection pressures that may impact prevalence.

P3-237 Employing Environmental Epidemiology Surveillance in Surface Water to Investigate Human-Environment Pathogen and Antibiotic Resistance Dissemination

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Introduction: The role of water quality is gaining importance in the spread of antimicrobial resistance (AMR). Freshwater bodies receive organic waste, including feces and urine, from various sources. This contamination circulates back to us when used for irrigating crops and for consumption by productive animals.

Purpose: Through the analysis of various projects related to our environmental epidemiology surveillance in surface water, our objective is to deepen our understanding of the dynamics of pathogen and AMR dissemination between humans and the environment.

Methods: Samples were collected from various environmental sources, including surface water for agriculture, drinking water, vegetables, and human hospital patients in central Chile. AMR Enterobacteria were isolated using MacConkey agar plates supplemented with Ceftazidime and another set with Ciprofloxacin. Colonies were selected based on morphology and identified with MALDI-TOF. Salmonella was isolated using XLT-4 agar, and fecal coliforms were quantified with standard methods. The AMR profile was characterized through Kirby-Bauer testing. Results were analyzed for statistical associations with environmental factors, heat maps, and phylogenomic reconstruction to assess potential isolates dissemination between humans, river water, and vegetables.

Results: Aquatic epidemiological surveillance is a valuable tool for detecting fecal contamination and studying AMR. Its implementation requires multidisciplinary collaboration. Our findings indicate the dissemination of AMR Enterobacteria between humans and the environment, though further research is needed to determine the directionality.

Significance: The isolation of AMR bacteria from all sampled water courses underscores the necessity to monitor these systems for a thorough understanding of environmental resistance. These environments could serve as sources for acquiring AMR microorganisms, posing a risk to public health.

P3-238 Food and Water Safety Practices and Preparedness of Ontarians During Power Outages

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❖ ***Developing Scientist Entrant***

Introduction: Power outages are becoming more frequent due to climate change, aging infrastructure, and high electricity demand. These events can lead to foodborne and waterborne illness risks for consumers if proper protective measures are not taken at home.

Purpose: The purpose of this study was to understand the behaviors and experiences of Ontarians related to food and water safety preparedness at home during power outages and floods.

Methods: A qualitative descriptive study was conducted, consisting of six virtual focus groups, each with eight people. The focus groups followed a semi-structured interview guide containing 16 questions on power outages, flooding, and information sources. Participants were selected from geographically dispersed locations that had experienced power outages due to weather events in recent years. Three groups were conducted with participants living in urban areas and three with participants from rural areas. Thematic analysis was conducted to identify key themes and trends using NVivo.

Results: In total, 46 Ontarians participated in the focus groups, with an approximately equal gender balance. Among the 46 participants, 19 (41%) were aged 30-39 years and they had varied educational backgrounds. Thematic analysis found that knowledge, resource availability, and trust in authorities were the primary factors explaining Ontarians' behaviors regarding food and water safety during power outages at home. For example, the majority of participants incorrectly believed that refrigerated foods were safe to consume after a 24-hour power outage. When discussing their behaviors, participants commonly talked about the need for alternate power sources like a backup generator or solar panels that were too expensive to install. Lastly, most of the participants expressed a great deal of trust in the information provided by governmental public health agencies.

Significance: Results will be used to make recommendations for improving risk communication with the public to reduce enteric illness risks during power outages.

P3-239 Treating Recycled Wastewater for Indirect Potable Use: A Pilot Membrane-based Approach in Westminster, MD

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Introduction: With over half of the world's population projected to face water scarcity for at least one month annually by 2050, there is a critical need to employ innovative water reuse strategies. The City of Westminster, Maryland has been experiencing intermittent droughts resulting in significant strains on its drinking water system.

Purpose: To alleviate this stress, the city is testing a multi-barrier membrane-based approach to treat and reuse recycled wastewater at what will be the first indirect potable reuse facility in the State of Maryland: PUREWater Westminster. Our study evaluated the microbial removal efficiencies of the membrane-based treatment chain.

Methods: Water samples were collected over six months from raw wastewater influent and after various treatments (microfiltration/ultrafiltration, reverse osmosis, granular activated carbon, and UV/ozone advanced oxidation) along the treatment chain at the wastewater treatment facility. Total bacterial community composition was characterized using metagenomic sequencing of genomic DNA on the NextSeq2000. *E. coli* and total coliforms were enumerated from all water samples using U.S. EPA standard membrane filtration method 1604.

Results: While *E. coli* and total coliforms were detected in raw wastewater samples, purification facility influent samples, and GAC concentrate samples, they were not detected in the final purified water. Mean *E. coli* and total coliform log reduction values through the entire wastewater treatment train were 7.81 (SD ± 0.09) and 8.97 (SD ±0.82), respectively. Although metagenomics data revealed the presence of several pathogenic bacteria (e.g., *Pseudomonas, Klebsiella* and *Salmonella)* in the raw wastewater and facility influent samples, the majority of samples collected further along the membrane-based treatment chain had no to very low levels of genomic DNA that precluded sequencing.

Significance: Our findings suggest that the pilot membrane-based treatment system is highly effective in removing bacterial contaminants from recycled wastewater. Our data will support the full-scale implementation of the membrane-based treatment system, enabling the City of Westminster to successfully alleviate its drinking water security challenges.

P3-240 Novel ZVI-Biochar-Mycoremediation Filtration Utilizing White-Rot Fungi *Phanerochaete chrysosporium,* to Inhibit Pathogenic Bacteria in Agricultural Water

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❖ ***Developing Scientist Entrant***

Introduction: Mycoremediation may increase microbial removal from water by filtration. Activation of white-rot fungi ligninolytic activity along with bacterial inhibition by biochar and zero-valent iron (ZVI), creates a novel filtration system to reduce pathogens from agricultural water.

Purpose: To evaluate removal of *E. coliTVS355* and *Salmonella* spp. by activated *Phanerochaete chrysosporium* (PC) grown on woodchips (WC) combined with ZVI and biochar.

Methods: Filters were built utilizing PC-treated WC (100g), 35%:65% ZVI:sand (100g) and biochar (BC) (100g). Filters were primed with 3 pore volumes (pvol) of artificial ground water (AGW) with a flow rate of 0.5L/min and retention time of 0.6min. Inoculated (3pvol of 5-log CFU/mL) AGW, with *E. coli*TVS355 or *Salmonella* spp. was pumped through the filter. Filters were flushed 2x with 2pvol of AGW. Effluent samples were collected after each run. Samples of each filtration matrix (WC, ZVI, and BC) were collected. *E. coli* was enumerated on MacConkey agar with rifampicin and *Salmonella* was enumerated on XLT4 agar. Control filters did not include PC. Chemical analysis included pH, and H_2O_2 concentrations for each filter. Data were analyzed using one-way ANOVA and student T-test across 4 trials (n=8 per/treatment).

Results: Bacterial removal was statistically significant in the presence of PC when compared to control (p<0.0092). PC-filters tested at 0.5mg/L H_2O_2 indicative of ligninolytic activity compared to none detected in controls. Log removal of *E. coli* by PC-filtration was 2.2-log CFU/mL and without PC was 1.2-log CFU/mL. After filtration, log removal of *Salmonella* with PC was 1.0-log CFU/mL and without PC, 0.1-log CFU/mL. Among filter matrices, BC retained significantly (p<0.0001) greater *E. coli* levels (1.9-log CFU/g) compared to WC or ZVI. Electron microscopy provided visualization of PC antagonism toward bacteria.

Significance: PC combined with BC and ZVI removed >4-log CFU/mL of *E. coli* and *Salmonella* from water, creating an advantageous filtration system for irrigation water.

P3-241 Zero-Valent Iron Sand Filtration Reduces *Cyclospora cayetanensis* Surrogates, *Eimeria tenella and acervulina*, in Water

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Introduction: Outbreaks of cyclosporiasis linked to fresh produce demonstrate the need to mitigate pre-harvest contamination. Sand filtration is commonly used to reduce levels of pathogens in irrigation water and adding zero-valent iron (ZVI) to sand improved disinfection of irrigation water in previous work.

Purpose: This study evaluated the efficacy of laboratory- and field pilot-scale ZVI/sand filters to reduce *Eimeria* spp. oocysts in irrigation water.

Methods: Laboratory-scale filters were constructed using PVC pipes (4.25 x 2-inch) filled with either 100% sand or 50% ZVI/50% sand. *E. tenella* oocysts ($5x10^5$) in 100 mL of sterile deionized water (DI) were filtered followed by a 1 L flush and 1 L backflush of DI. Oocysts were enumerated from filtered effluent and backflush water. Trials with sand and ZVI/sand filters were performed in triplicate. Field pilot-scale filters were constructed by filling a commercial sand pool filter with 35% ZVI and 65% sand. *E. acervulina* oocysts ($2x10^7$) in 10 L of municipal water (MW) were pumped through the filter and flushed with 10 L MW. The 10 L inoculation and flush were repeated once before backflushing with 10 L MW. Oocysts in the effluent and backflush waters were concentrated and counted using McMaster chambers.

Results: Laboratory-scale ZVI/sand filters reduced 99.9% of *E. tenella* oocysts, whereas sand filters reduced 55% of oocysts in water. In preliminary field

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pilot-scale filter trials, *E. acervulina* oocysts were reduced by 64% after each inoculation/flush event. Between 4 and 9% of oocysts were recovered in back-flush water from both filters tested.

Significance: Differences in water type, filter design, *Eimeria* species, and ZVI percentage influenced differences in laboratory and pilot-scale results. These findings demonstrate ZVI/sand filtration of irrigation water can reduce *C. cayetanensis* surrogates in irrigation water.

P3-242 Surveillance of Non-bacterial Pathogens and Indicator Organisms in Agricultural Water Using Digital Polymerase Chain Reaction

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✦ ***Undergraduate Student Award Entrant***

Introduction: Agricultural water can be a cause of foodborne outbreaks. Surveillance of water for non-bacterial pathogens and indicators can provide information on human and environmental contamination.

Purpose: This study seeks to address detection of viral foodborne pathogens and indicators in water sources and identify means to correlate these.

Methods: Digital polymerase chain reaction (dPCR) was utilized to detect non-bacterial pathogens and indicator organisms in water samples (1L), that were collected from farms across two different geographical areas with intensive agriculture (n=18 per area). Samples were ultrafiltered and ultracentrifuged for 45min at 3,000xg. Concentrate was collected and extracted with Qiagen RNeasy PowerMicrobiome or Qiagen DNeasy Powersoil kit. dPCR for hepatitis A virus (HAV), norovirus GI & GII (NoV), pepper mild mottle virus (PMMoV), *Cyclospora,* CrAssphage, and *Bacteroides* results were obtained and described by percent and average concentration per area. Statistical analysis was performed with one-way ANOVA.

Results: HAV concentration was statistically different (p=0.0003) between areas, with 28% of samples in area-A and 44% in area-B, average of 2.665 copies/µL-4.702 copies/µl. NoV GI concentration was similar (p=0.7143) in both areas, 17% of samples in area-A and 28% in area-B, average concentrations of 2.825 copies/µl-2.971 copies/µl. Indicator detection did not match that of pathogens. No *Cyclospora* or NoV GII was detected in these samples. PMMoV concentration was statistically significant (p=0.0055), detected in 6% of samples in area-A and 28% in area-B, an average concentration of 21.96 copies/µl-2.965 copies/µl areas A and B. CrAssphage was detected at lower frequency in area-A (17% at 2.948 copies/µl) than area-B (22% at 10.30 copies/µl), both significantly different (p=0.0134). *Bacteroides* was detected in samples from area-A only, in 50% of the samples at an average of 3.692 copies/µl.

Significance: Pathogen presence in water and subsequently public health may be impacted by temporal and other characteristics. Pathogen detection remains challenging and indicators have limited value in the detection of viral pathogens in agricultural water.

P3-243 Development of a Portable Nanozyme-Based Sensor for the Detection of Fecal Contamination of Agricultural Water

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Introduction: Fecal contamination of agricultural water is a significant food safety risk. There is an unmet need to develop sensors that can rapidly detect biomarkers of fecal contamination in agricultural water with high sensitivity for field applications.

Purpose: This work was focused on the development of a portable electrochemical sensor for rapid and on-site detection of bile acids as non-living indicators for the fecal contamination of agricultural water

Method: The nanozyme-based sensing chip was fabricated based on an assembly of platinum nanoparticles embedded in a hierarchical metal-organic framework (MOF) as a sensing matrix on a disposable screen-printed chip. The electrochemical characterization of the developed MOF-based electrochemical chip was carried out using electrochemical impedance spectroscopy and cyclic voltammetry. The analytical performance, including dynamic detection range and limit of detection of the fabricated chip for detecting lithocholic acid (as a model bile acid) was measured. The applicability of the developed sensor was examined using agricultural water spiked with different concentrations of bile acids.

Results: The 3D hierarchical structure of a conductive MOFs matrix increased the effective electroactive surface area by about 8-fold compared to a conventional MOF matrix. The MOF-based chip exhibited high sensitivity for the detection of lithocholic acid with a dynamic range of 0.01 to 5 µg/mL and a detection limit of 0.0084 µg/ml. The fabricated chip showed robustness and reproducibility for 20 repeated electrochemical measurement cycles. Furthermore, the established nanozyme-based sensor enabled the detection of spiked bile acids in agricultural water without any pre-treatment steps and within a detection time of 1 minute using a handheld device.

Significance: This work illustrates the development of a sample-to-answer chip for rapid and highly sensitive monitoring of fecal contamination in agricultural water to improve food safety.

MP-01 Produce TRAINer – University of Maryland College Park and the University of Maryland Eastern Shore

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Introduction: An interdisciplinary team implemented a needs assessment (2022) among small and medium-size growers to identify gaps regarding worker training as it applies to The Food Safety Modernization Act Produce Safety Rule (FSMA-PSR).

Purpose: The purpose is to share and walk interested groups through our worker-informed, interactive farm training website toolkit (non-internet version available too), consisting of: 1) an introductory video on risk assessment; 2) a worst-case farm scenario; 3) building visual and printable SOPs; and 4) a this-or-that risk assessment through a harvesting day. Additionally, we would like to showcase our supervisor Train-the-trainer manual to assist with effective worker training.

Methods: During the development process, the team worked with growers across Maryland to receive their feedback on the direction of the materials. This was done via farm visits and during breaks at various state-wide training engagements; FSMA-PSR, cleaning and sanitizing, and standard operating procedure workshops. Lastly, subject matter experts (SMEs) were invited to review the materials using a Health Literacy Rubric. SMEs represented Extension, private consultants, industry, SNAP-Ed, farm workers/managers, and beginning farmers. The materials were styled to complement our collaborator's previous work with "Produce Safety Matters" (2010).

Results: Four lessons with a total of six activities and a supervisor manual were developed to aid in workers' understanding of risk assessment to improve their in decision-making processes during a typical day on a produce farm. The majority of the activities focus on encouraging group discussions and highlighting "WHY" this behavior is important to public health, a best practice for adult education.

Significance: To our knowledge, this is the first online, as well as in-person training toolkit that incorporates a video on risk assessment applied to produce farms, visual SOPs, and a supervisor manual that helps break down each lesson, as well as goes into adult learning and food safety culture, that can improve and retain worker training on identified FSMA-PSR gaps.

MP-02 In-Home Freeze Drying: Principles, Equipment, and Best Practices for Food Safety

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Introduction

In-home freeze drying is gaining popularity as interests in consuming raw or minimally processed foods and preserving foods have increased among consumers. Recently, purchasing a pilot- or kitchen-scale dryer has become affordable. However, freeze dried foods are potentially hazardous foods as they can be contaminated during preparation, equipment loading, packaging and rehydration, and most consumers lack adequate knowledge of the freeze-drying process to adequately control their freeze dryer, monitor final water activity, and determine corrective actions.

Purpose

As part of Nebraska Extension's Food Processing for Entrepreneurs Series, extension materials have been developed to provide guidance on the fundamentals of the freeze-drying process and adequate equipment and process controls for microbial food safety to cottage food producers, entrepreneurs, and enthusiasts.

Scope of the Extension Materials

The materials will cover the following topics:
principles of freeze drying and sublimation process,
equipment parts, controls, maintenance, and sanitation,
typical operating procedures and conditions (plate and product temperatures),
process controls for food safety (pH, initial and final aw),
corrective actions should a freeze drying cycle fail, and
packaging considerations for long-term storage.

The types of foods suitable for freeze drying – with careful attention given to foods typically allowed by cottage food laws, which vary by state – and potential rehydration issues related to product quality and food safety are also discussed.

Significance

These materials provide guidance to food entrepreneurs, food safety extension personnel, and food inspectors interested in learning more about the process of making safe, high quality freeze-dried foods.

MP-03 Parameters that Determine the Risk of Pathogen Growth and Survival in Natural Cheeses (>39 to 50% Moisture) Made from Pasteurized Milk: Literature Review and Analysis

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Introduction: A group of industry and academia professionals, in collaboration with the International Dairy Foods Association, is developing a tool for the dairy industry to further identify the factors including but not limited to pH, salt and sodium content, competitive lactic acid cultures and other metabolites, and water activity, that contribute to microbial safety of intermediate moisture cheeses made from pasteurized milk.

Purpose*:* A key objective of this project is to collect and analyze available data on pathogen growth in cheeses of intermediate moisture (>39% to 50%) made from pasteurized milk to better understand the risks of these cheeses to develop a tool to be used by industry and regulators.

Methods*:* The scientific literature and government and trade organization documents (peer-reviewed or equivalent, such as cheese industry publications or regulatory documents) will be examined to identify studies investigating the potential of intermediate moisture cheeses to support pathogen growth or survival. Microbial pathogens that will be covered include *Listeria monocytogenes* (and its surrogate, *Listeria innocua*), *Salmonella* spp., *Staphylococcus aureus* (growth and toxin production), and pathogenic *Escherichia coli.*

Results*:* The output of this project will be a tabular summary of peer-reviewed studies and a peer-reviewed document.

Significance: Analyses of these data may demonstrate that certain intrinsic factors, manufacturing methods, storage conditions, etc., consistently prevent pathogen growth or survival in the cheeses. These data are expected to help identify knowledge gaps in cheese categories and prompt future research on risks of cheeses. It will also provide the dairy industry with a peer-reviewed technical resource that will enhance their product and process risk assessments. Further, this work may help inform future federal regulatory policies including defining more granularly the cheeses that should be subject to the FDA's food traceability list when that list is next updated and the agency's voluntary sodium reduction targets.

MP-04 A Website-Based Interactive Analysis Tool Enabling Exploratory Data, Statistical and Machine Learning-Based Analysis of Microbiota Datasets for Food Safety and Quality Applications

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Introduction: Analysis of food and associated environmental microbial communities is generating novel insights for the improvement of food safety and quality and adoption of these advancements in the field can be further augmented by the availability of easy-to-use web-based data analysis tools.

Purpose*:* Develop a web-based free microbiota data analysis tool (post-sequencing and microbiota data generation by bioinformatics analysis) as most currently available resources are either commercial versions or require programming or coding skills to analyze the data before making any interpretation.

Methods*:* A website incorporating standard microbiota analysis packages was developed with an interactive user interface. In addition, to identify a set of microbiota features that associate with a certain research question or study metadata, a novel workflow comprising statistical methods and Machine Learning algorithms was deployed on the website. This approach has certain key stages namely (1) Data engineering (2) Machine Learning based Modeling (3) Performance Evaluation metrics and (4) Visualizations.

Results*:* A website-based analysis tool was developed to (i) enable exploratory microbiota data analysis and (ii) perform Machine Learning analysis to identify microbial features that potentially associate with a research question/ study metadata. The website provides certain in-depth functionalities such as alpha diversity, beta diversity, differential abundance analysis, feature reduction, feature selection, classification-based modeling, model feature exploration and their importance. A preliminary analysis using the default settings is proposed as a starting point for the microbiota analysis. The github link to the website package is https://github.com/ZakariyaTaha/Mcb_Website.

Significance: The workflow and methodology presented here through a free, interactive web-based tool can be applied to various microbiota datasets to identify signals that associate with a research question/ study metadata. Importantly the website allows domain experts with limited or no programming knowledge to efficiently collaborate with bioinformaticians and/ or data scientists during the analysis of microbiota datasets.

MP-05 The USDA NAL Food Safety Research Information Office (FSRIO): A Key Information Product

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USDA NAL Food Safety Research Information Office (FSRIO), Beltsville, MD

The USDA National Agricultural Library is one of five national libraries across the United States. It has a unique information center for the food science community entitled the Food Safety Research Information Office (FSRIO). The conference presentation will provide insight into the digital resources for investigators of food science in academia, industry, and the government. Further, there will be a discussion about how to explore its key information products on Bacterial Pathogens and Viruses, Chemical Contaminants, Aflatoxins, and other publicly accessible research information. Its flagship information products include a unique automated Research Projects Database and Research Publication application for only food science experts and students. Its

food safety news and current events are available on the main webpage and reach domestic and international stakeholders who are food safety professionals and members of the International Association of Food Protection (IAFP).

MP-06 CDC Resources on Safer Food Choices to Avoid Food Poisoning

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CDC, Atlanta, GA

CDC developed a series of educational resources on foods and beverages that pose a higher risk of contamination with foodborne pathogens and safer alternatives. These resources were created to fill a gap in our current communication portfolio. Previous materials were pathogen-specific, like a factsheet on how to prevent *Listeria*. Through our consumer research, however, we found that members of our audience were interested in having one resource that listed riskier foods for all foodborne pathogens and safer alternatives to consume. To create these resources, we segmented our market to reach demographic groups at greater risk for severe food poisoning, including *adults aged 65 and older*, *people with weakened immune systems*, *pregnant people*, and *children under the age of 5*. We created resources for each demographic group that included riskier foods and beverages specific to their population and safer food and beverage alternatives. We also created a similar resource for *general consumer*s because it is important for everyone to be aware of what foods may put them at increased risk for foodborne illness. We incorporated graphics that represented in-group diversity based on results from previous message and material testing. On *our site*, we included information about why each demographic group is at increased risk for severe food poisoning. This was information our message testing participants felt was lacking in our previous resources. We had the opportunity to test a sample of these resources in our most recent market research and participants appreciated the choices they were presented with and felt that the resources gave them a sense of agency over their health.

MP-07 The National Center for Home Food Preservation Launches Kombucha Fermentation Toolkit to Meet Growing Demand for Home Food Preservation Education

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Introduction: The National Center for Home Food Preservation (NCHFP) has developed a toolkit designed to assist Extension agents and educators in conducting county-based programming focused on fermentation, with a specific emphasis on kombucha.

Purpose: The initiative addresses a growing interest in fermentation techniques among the public and aims to equip agents with the necessary resources to deliver effective educational programming.

Methods and results: The toolkit comprises various components to facilitate the implementation of fermentation workshops and classes. These include a detailed lesson plan outlining the structure and content of the program, a validated recipe for brewing kombucha at home, an evaluation tool to assess program effectiveness and promotional materials such as class advertisement flyers. Additionally, the toolkit includes FAQs tailored for both the general public and agents, providing answers to common questions about fermentation and kombucha. To further support agents in promoting their programming efforts, the toolkit offers examples of a newspaper article and Radio Public Service Announcement (PSA), which can be customized to include agent details and disseminated through local media channels. It enhances the visibility of the fermentation program within the community and encourages participation from interested individuals.

Significance: The development of this toolkit addresses a notable gap identified through the NCHFP, where requests for tested fermentation recipes, particularly for kombucha, have been consistently received over the past two years. By providing agents with validated resources and educational materials, the NCHFP aims to meet the demand for reliable information and instruction in home food preservation practices related to fermentation. Through this program, participants can learn about the science and techniques behind fermentation while gaining practical skills to safely produce kombucha at home.

MP-08 Determine Shelf Life and Food Safety of Meat with DMRI Predict 2.0

Gry Dawn Terrell
Danish Meat Research Institute, Taastrup, Denmark

Introduction: DMRI Predict is a collection of predictive models that can be used to assess food safety and spoilage, both microbiological and sensory, of meat and meat products.

Purpose: Changing needs in the industry called for updated functionality, prompting reinventing the concept of the model collection, and resulting in a refreshed and uniform user interface and several new features.

Method: The models have been developed in collaboration with the meat industry and are based on a large number of shelf-life studies and challenge tests carried out in meat.

Results:

The new features

'My products' is a personal product library, where the user can enter relevant parameters for the product, save them and access them later. Not only is it a timesaver to be able to pick up where you left off, but it also enables the user to work with multiple models simultaneously and run all the relevant predictions with a single click.

'Guide me' is a risk-based approach to the models, which helps the user to identify the models that are relevant to use based on the bacteriological risks associated with the product in question.

Other features, focusing on ease-of-use have been added as well.

DMRI Predict has been transferred to a new platform, which has simplified adjusting and expanding current models. In 2024, the ConFerm model is expanded to include products with a lower salt content and two brand new models, growth of gas-producing lactic acid bacteria in cold cuts and shelf life of by-products (hearts, liver, diaphragm), are added.

Significance: With around 2,000 registered users worldwide, DMRI Predict plays an important role in ensuring that meat and meat products around the world are produced safely and with a reliable shelf-life determination. DMRI Predict is completely free to use and can be accessed on *www.dmripredict.dk*.

MP-09 Cheese Milk Thermization App

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Introduction: Certain cheeses can be legally produced in the United States using raw milk but must be aged for at least 60 days to reduce pathogen risks. However, even when aged for 60 days, Shiga toxin-producing *Escherichia coli* (STEC) can survive in certain hard cheeses and *Listeria monocytogenes* can grow in soft cheeses. Thermization, as a sub-pasteurization heat treatment, is used to reduce the risk of pathogens in raw cheesemilk while retaining some quality attributes in the cheese.

Purpose: To provide cheesemakers with a tool to determine heating required to reduce STEC and *L. monocytogenes* in raw cheesemilk.

Methods: D- and z-values for STEC and *L. monocytogenes* derived from published experiments in whole bovine milk were incorporated into a web-based app to determine the milk hold time and temperature necessary for log-reductions of the two foodborne pathogens.

Results: The app is accessible via the University of Wisconsin-Madison Food Research Institute website at https://fri.wisc.edu/resources_thermization.php. Cheesemakers can use the drop-down menus to select bacteria of choice (*L. monocytogenes* or STEC) and desired log-reduction of the chosen bacteria

Poster

(3-, 4-, 5-, or 6-log kill). A temperature range of 140°F to 155°F (60°C to 68°C) in whole degree increments is included in the application. Outputs are "Hold Time" in seconds and corresponding log-reduction of the unselected pathogen according to the temperature selected. Values are valid for bovine milk with typical fat content 5% fat or less; milk of higher fat content may require greater thermization. Temperature must be taken at the coldest point of the system and milk must be stirred/agitated during thermization to ensure each particle is heated. Time output is for hold time and does not include integrated lethality for come-up and cool down times.

Significance: This thermization app should be used in conjunction with Good Manufacturing Practices to enhance the safety of cheeses made with unpasteurized milk.

MP-10 Guides, Classes, and Tools for Processors, Extension, and Educators

Timothy Stubbs

Innovation Center for U.S. Dairy, Rosemont, IL

The non-profit Innovation Center for US Dairy Food Safety Team convenes food safety experts from processors and academia to share best practices. In this session we will share resources we've developed for processors as well as videos, guides, online courses, and websites developed with small/new processors in mind. We invite processors as well as extension professionals, regulators, and others who work with processors to leverage the free resources at *www.usdairy.com/foodsafety*

MP-11 The Food Safety Resource Clearinghouse: Redesigning a Crowd-Sourced Food Safety Information Repository

Annie Fitzgerald

University of Vermont, Burlington, VT

Introduction: The Food Safety Resource Clearinghouse is an online curated and crowd-sourced collection of Produce Safety Rule (PSR) and Preventive Controls for Human Food (PCHF) Rule resources related to the Food Safety Modernization Act (FSMA), managed by the Northeast Center to Advance Food Safety (NECAFS). Resources, e.g. published rules, national curricula, fact sheets, webinars, supplemental educational materials, and videos have been and are being developed by partners in research, education, outreach, and regulatory roles. Given this breadth of work, it can be challenging to learn about all the resources that exist and where to find them. The Clearinghouse is a one-stop-shop of resources that collects, curates, and connects users through search functionality to relevant FSMA-related materials by authors across the nation in one single site.

Purpose: As the Clearinghouse has grown, the need to update the search and content display functionalities has emerged. With funding from the United States Department of Agriculture Food Safety Outreach Project, NECAFS has launched a review and will be updating the Clearinghouse to incorporate data-driven decision making and improve content accessibility.

Methods: NECAFS will use data collected from Google Analytics and user feedback to conduct an analysis of information seeking behavior and resource gaps. NECAFS will then develop a more responsive search feature and revise the data architecture to create links between related content and build an information taxonomy to guide users through complex topics.

Results: This new initiative will improve access and create more learning opportunities for stakeholders.

Significance: The goal of the Clearinghouse is to connect food safety people, projects, and publications across the nation through a verified process so that regulators, educators, technical service providers, growers and processors can feel confident knowing that the information is from a trusted source of food safety related information.

MP-12 PAS 320 – A Free Resource for the Industry on Developing your Food Safety Culture

Alison Cousins

BSI, London, England

Culture will eat your food safety management system for breakfast! You have all the processes and procedures set up for your food safety management system, but when you put everything into place it is **people** that will oversee, manage and comply with it. For every product recall and food safety incident there is a person (or people) who has acted in a way that compromised your product.

It is widely recognized that the culture of an organization is key to the success of implementing food safety management standards and schemes, such as HACCP, ISO 22000, FSSC 22000, BRCGS and SQF. If this organizational culture does not prioritize and support food safety, then it will not be possible to implement these standards effectively. And it won't be possible to consistently deliver safe food products to customers. GFSI published a Position Paper on Food Safety Culture in 2018, and now mandates that all GFSI-recognised schemes include requirements for organizations to mature their food safety cultures.

BSI brought together leading experts on culture to publish ***PAS 320 Developing and sustaining a mature food safety culture – Guide*** in March 2023. A PAS is a Publicly Available Specification – it is a 'how-to' guide that gives a framework for industry on best practice. It is written in a format that gives clear guidance for teams, and is free to download and use by anyone who works within a food organization – large or small. It will help you and your organization to meet GFSI and regulatory requirements on food safety culture.

Link to download PAS 320 Developing a Mature Food Safety Culture - PAS 320:2023

Use the QR code given below to go to the BSI website and download PAS 320

MP-13 Eye on Food Safety with Dr. D

Tina Brillinger

Global Food Safety Resource Centre Inc., Toronto, ON, Canada

Eye on Food Safety with Dr. D is a monthly food safety podcast produced by Global Food Safety Resource (GFSR) and hosted by Dr. Darin Detwiler who engages with international food safety experts through a Q & A format discussion while engaging with our live audience. GFSR produces these live on LinkedIn through GFSR's LinkedIn group but is now widely available on Spotify with archival podcasts from 2023. Range of Topics have included: Resiliency (this month) Digitizing Food Safety: Food Watch, Cybersecurity, Food Safety & Sustainability, Sanitation from the top Down, The Evolution of Traceability, Navigating Foodborne Outbreaks and more!

MP-14 Food Safety on Subsistence Farms in Nepal and Uganda

David Blomquist

DFB Consulting, Hastings, MN

Food safety is something that is a vital part of any food manufacturing and distribution in the world. While the principles and outcomes of food safety are the same, the techniques, tools, and approaches to achieve food safety for it requires a completely different set of procedures when the manufacturing process is on subsistence farms in developing countries.

Nepal is a country with a set of challenges that are not common in most areas of the world. Milk-producing farms are small and located in valleys. Heavy rains can make roads impassable, making it impossible to deliver the milk to a processing facility consistently. The loss of a day's production is a significant problem during the rainy season as it is repeated many times during the season. A project sponsored by the US Agency for International Development

(USAID) and Catholic Relief Services (CRS) led to producing direct acidification mozzarella cheese on the farm. The cheese gives longer shelf life allowing a better chance to get the product to a market as well as getting a higher price for their value-added cheese. The identification of food safety techniques, training and tools used to and development of and achieve an effective food safety system and products safe for the consuming public.

Uganda is another example as it has slightly different issues. Milk production is also from small subsistence farmers where the largest food safety challenge is refrigeration to slow or prevent spoilage. Periodic electrical grid failures cause milk to spoil. Transporting it to a central receiving station with more reliable refrigeration capability is accomplished via walking, bicycling, or on a motorcycle using plastic or metal cans. Procedures and techniques that address the challenge of refrigeration as well as for cleaning and sanitizing of storage and transport containers and vessels will be reviewed.

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Developing Scientist Competitors

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