ABSTRACTS

A collection of abstracts from the IAFP 2023 Annual Meeting

Advancing Food Safety Worldwide®

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IVAN PARKIN LECTURE

SUNDAY, JULY 16
OPENING SESSION
6:00 P.M. – 7:30 P.M.

ANATOMY OF A STANDARD

SARAH CAHILL, PH.D.

Food and Agriculture Organization of the United Nations
Rome, Italy

SUNDAY, JULY16
OPENING SESSION
6:00 P.M. – 7:30 P.M.

My journey with international standards and Codex standards in particular started almost 25 years ago, not with the standards themselves but with the science that underpins them. Along that journey, I became aware of the intricacies that make up a standard.

Standards are part of all of our lives, whether it is the standards we expect the products and services we use to adhere to, or the standards we set for ourselves. But if we analyze and dissect a standard, if we peer into its anatomy, what might we find and why is a particular standard the one that is accepted?

For 60 years, an international body known as the Codex Alimentarius Commission has been bringing countries and organizations together to develop global standards for food safety and quality. Hundreds of standards, guidelines, and codes of practice, and thousands of quantitative standards like maximum levels for contaminants and food additives, and maximum residue limits for pesticides and veterinary drugs in food have been developed. This is an impressive output and one with which a recent survey of Codex Members suggested a good level of satisfaction. However, these standards are highly variable in content, structure, and the way in which they would be applied. So, can they all be equally satisfactory? Could we say that there is a common anatomy for a successful standard?

Science and cumulative evidence are the heart of any food safety standard. International scientific committees existed before Codex. When critically assessed and reviewed science is available, standards can be developed when they are needed, especially in response to food safety crises. The standard for melamine in food or the code of practice to minimize the risk posed by *Cronobacter* spp. in powdered formula are both fine examples from Codex.

But sometimes those setting standards are unable to agree. The complexity of the issue requires deep thinking before the standard setting discussion can be concluded. Our intimate relationship with food and cultural preferences or practices can influence or drive decisions on standards and even challenge what the science is telling us. No matter how good the scientific work, the confidence and trust of those taking the decisions, the people that represent the countries and users of the standards are key. Successful engagement of these people is the blood, the connective tissue, that brings together the various parts – that is to say the science, the acceptance of that science, the consideration of cultural preferences, among others. These are the building blocks that determine whether a standard comes to life or not.

A standard only achieves its goal if used. Keeping the application and required impact to the forefront of standard setting means those working on the operational front of the food system – the nerve center for food safety – as well as those representing consumers, have to be part of the discussion.

Standards, or more likely the lack of them, impact most of us without realizing it. Long before I even knew of Codex, I saw the impact of foodborne disease on family members, and when I worked in quality assurance labs, swabbing surfaces for environmental monitoring or testing milk for antibiotic residues, I was in reality on the front line implementing standards that had been agreed many miles away. The same can be said for any food safety practitioner; without their voices and hands a standard either doesn't exist or simply gathers dust.

Science, people, and practices are all necessary to bring a food standard into the Codex Alimentarius. Dissecting and analyzing an array of Codex standards, I will try to shed light on why they are still alive and kicking after 60 years, and what their future might entail.

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JOHN H. SILLIKER LECTURE

WEDNESDAY, JULY 19

CLOSING SESSION

4:00 P.M. – 4:45 P.M.

RANDY HUFFMAN
Chief Food Safety and Sustainability Officer
Maple Leaf Foods
Mississauga, Ontario, Canada

RANDY HUFFMAN

Dr. Randall Huffman (Randy) is Chief Food Safety and Sustainability Officer at Maple Leaf Foods. His role encompasses leadership of Food Safety and Quality, Occupational Health, Safety and Security, Environmental Sustainability and Animal Care.

Randy leads a team that has developed and is executing world class strategies to deliver on Maple Leaf Foods' commitments to produce safe, great tasting food produced in a safe work environment and to become the most sustainable protein company on earth. Maple Leaf Foods' commitment to become a global leader in Animal Care and to reduce greenhouse gas emissions from the company's operations to levels in line with the Science Based Targets Initiative has given us clear and very bold targets to achieve.

Randy joined Maple Leaf Foods as Chief Food Safety Officer in January 2009 and during his tenure has had accountability for several functional areas of the business. In 2011 he assumed leadership of Six Sigma and Food Quality. In 2014, he was appointed Senior Vice President, Operations, in addition to his role leading Food Safety and Quality. In the following 3 years, he led Manufacturing across 12 prepared meats plants as well as Corporate Engineering, Manufacturing Services, Occupational Health and Safety, Security and Environment, and the Operations Excellence and Learning teams.

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Symposium Abstracts

S1 Poultry Sampling Symposium – The Path to Improved Poultry Safety through *Salmonella* Assessments

WILLIAM SHAW: *USDA Food Safety and Inspection Service, Washington, DC, USA*
ASHLEY PETERSON: *National Chicken Council, Washington, DC, USA*
TERRANCE ARTHUR: *U.S. Department of Agriculture – ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*

As part of the effort to reduce illness associated with poultry, the USDA and FSIS propose to classify *Salmonella* as an adulterant in frozen, raw, breaded and stuffed chicken products with a quantitative standard of 1 CFU (viable cell) per gram. When these regulations are implemented, it is reasonable to expect the need for improved sampling techniques and quantitative *Salmonella* assays that provide measures of virulence. These needs will extent to other poultry products. Improved sampling and an improved assays are technically feasible but are still in development and will require validation. The industry needs procedures to be fast, accurate, easy to use and economical because the number of poultry tests is expected to increase greatly.

Moving forward, this increased level of testing must not be viewed as a mitigation. It is an assessment tool that will guide constant improvement efforts to reduce the levels of *Salmonella* and other pathogens in poultry. It will be important to share learnings for this industry to meet the new proposed standard. USDA will play a critical role in this sharing.

Present sampling methods for many poultry products are under review. Aggregated sampling techniques similar to those used in beef are under study. Validation results will be shared. These techniques will reduce labor and provide non-destructive sampling. The goal being to provide a more comprehensive and efficacious approach than the current methods.

The virulence of *Salmonella* is variable. The USDA proposal requires specifically identifying some of the more prevalent *Salmonella* strains in a sample. There are many approaches that can be considered. Their strengths and weaknesses will be considered with recommendations for achieving a suitable method.

Existing culture-based tests do not meet the proposed requirements because quantitation information is lost during the enrichment. MPN approaches will be slow and costly. Concentration of the signal is where solutions will be found. Several concentration approaches will be reported.

S2 Parasites and Virus of Fecal Origin in the Environment: New Perspectives for Detection and Surveillance

LIA STANCIU: *Purdue University, West Lafayette, IN, USA*
KALMIA KNIEL: *University of Delaware Department of Animal and Food Sciences, Newark, DE, USA*
RACHEL CHALMERS: *Public Health Wales, Microbiology and Health Protection, Singleton Hospital, Swansea, United Kingdom, United Kingdom*

Foodborne and waterborne parasites and viruses are recognized as primary etiological agents of disease and represent a serious threat to human health worldwide. Parasites and viruses such as norovirus, hepatitis A virus, *Crytosporidium* spp., or *Cyclospora cayetanensis* have been consistently found on fresh fruits and vegetables. The fact that these organisms have low infectious doses, are ubiquitous and highly resistant to the sanitation procedures commonly used in the food chain contribute to them being of health risk to consumers. These pathogens of mainly human fecal origin can be introduced to fresh produce either during pre- or postharvest activities. Various sources and routes of contamination can exist during any stage from farm to table and include harvest, transport, storage, sale, and distribution of fresh fruits and vegetables. Irrigation water, type of fertilizer, and presence of animals, and direct contamination from farm workers are the main pre-harvest sources of contamination. Abundance, diversity and proper identification of these pathogens in the environment is therefore crucial to be able to develop control strategies. In view of a changing ecosystem through climate change, management practices are continuously changing to adapt to the dynamics of this microbial community that are also constantly evolving. In line with the One Health concept, we need to examine all possible sources and routes of contamination and make sure we have the proper tools to quickly and accurately, detect viruses and parasites. This symposium will bring together experts from the field to address the need to (1) do surveillance studies across all diverse environmental sites, (2) evaluate and interpret surveillance information, and (3) identify analytical challenges and new approaches discovered along the way. Regulators and public health practitioners are starting to be aware of the existing and emerging risks of these pathogens to be prepared and respond accordingly.

S3 Beef Quality and Food Safety in the Canadian Beef Industry

REYNOLD BERGEN: *Beef Cattle Research Council, Calgary, AB, Canada, Canada*
CASSIDY KLIMA: *Beef Cattle Research Council, Calgary, AB, Canada, Canada*
NICK HARDCASTLE: *Cargill, Inc., Witchita, KS, USA*

Beef quality and food safety are pillars for sustainable beef production in Canada. Beef quality has been a key research priority in past decades, with the Canadian National Beef Quality Audit used to evaluate the quality, consistency, and competitiveness of Canadian beef by tracking performance and assigning economic values to quality defects or non-conformities. The information obtained by audits provide both a benchmark and target for quality levels, identifies areas for correction, and informs better management practices. Past audits have also evaluated the eating quality of Canadian beef based on metrics of importance to consumers (e.g., flavour, juiciness and tenderness). Similarly, research-driven improvements in food safety supports consumer confidence, advances technologies that keep Canada at the forefront for safe food production globally and ensures continued access to export markets.

In Canada, beef quality and food safety research are supported by cattle producers through Canadian Beef National Check-Off funds directed to the Beef Cattle Research Council (BCRC). The BCRC is Canada's national industry-led funding agency for beef, cattle, and forage research, and is guided by the Canadian Beef Research and Technology Transfer Strategy. Ongoing evolution in the sophistication of both industry production and marketing practices as well as the tools and technologies available to researchers necessitate the periodic re-evaluation of how beef quality and food safety research programs can be best aligned with current and expected future industry needs. The objective of this symposium is to discuss the status of beef quality and food safety research in Canada today and to identify where needs in these areas are arising and what challenges exists in advancing research moving forward.

S4 Novel Approaches to Monitoring Agricultural Surface Water Quality

REBECCA L. BELL: *U.S. Food and Drug Administration, Center for Food Safety & Applied Nutrition, College Park, MD, USA*
ZEYNAL TOPALCENGIZ: *University of Arkansas, Fayetteville, AR, USA*
MANAN SHARMA: *USDA ARS Environmental Microbial and Food Safety Laboratory, Beltsville, MD, USA*

Agricultural water is defined by the Produce Safety Rule as water used during the growing, harvesting, packing, or holding of covered produce. Untreated surface water (water that does not receive a disinfection treatment) sources constitute a high microbial risk to contaminating produce as they are often susceptible to fecal contamination through agricultural runoff or external sources. Prior studies also indicate that even ground water sources can be susceptible to fecal contamination from nearby livestock operations. Consequently, agricultural water for pre-harvest activities (irrigation, crop sprays, fertigation, etc.) can be a major pathway for the transfer of water-associated foodborne pathogens to fresh fruits, and vegetables. In recent years, the development of novel, multidisciplinary approaches have facilitated our understanding of the complexities of water source contamination in the pre-harvest environment, and its implications in the production of fresh produce. The purpose of this symposium is to provide the audience with recent advances in such approaches to measuring agricultural water quality through the lens of a pre-harvest food safety perspective. The sessions will be beneficial to academic, government, and industry professionals as well as extension agents and growers interested in its practical applications. The symposium will not only discuss new methods for collecting water quality data, but also providing contextual analysis through a variety of approaches to data gleaned from irrigation water environments.

S5 Latest Developments in International Organizations Making Food Safety Improvements and Successes Measurable

CAROLINE SMITH DEWAAL: *Global Alliance for Improved Nutrition (GAIN), Washington, DC, USA*
JEFFREY LEJEUNE: *FAO, Rome, Italy, Italy*
SIMONE RASZL: *World Health Organization, Geneva, Switzerland, Switzerland*

Food safety and quality are increasingly at the hearts and minds of society and regulators around the globe and improvements typically focus on capability and capacity building of nations and key stakeholders such as governments, consumers, industry and academia. Many governance models have been developed and implemented, but an overarching challenge is how to confidently measure real progress and improvement in food safety and quality or nutrition for consumers.

Food safety indicators are important to compare the effectiveness of food safety improvements across the globe, to help guide national governments with limited resources. In the complex context of food security and sustainability, several governmental, intergovernmental and non-governmental organizations have developed frameworks that include food safety indicators for quantitatively or qualitatively assessing the outcomes of interventions. In addition to measures of health impacts, food safety indicators should be feasible, cost-effective and easily measurable.

Such frameworks and metrics are typically designed and selected with respect to the scope and responsibility of individual international organizations, but they may contain common principles and indices that are important to identify and that help harmonize approaches to measuring food safety advancements at a more international or multi-sectorial level.

This short symposium will provide the audience with examples of the frameworks and food safety indicators developed or being developed by three different international organizations and an opportunity to discuss ambitions and learnings with the presenters.

The session is designed for food safety professionals interested in learning about the approaches and metrics that are being used or considered to monitor the outcome of food safety strategies and interventions at the national level. For example, categories of indicators include health impacts; core competencies for national regulators; and those used by consumer organizations and the food industry.

S6 Not Your Grandfather's Biofilm – What are Dry Surface Biofilms, and Why are They More Deceptive Than Their Good Ol' Slimy Counterparts?

JEAN-YVES MAILLARD: *School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, United Kingdom, United Kingdom*
MICHELE SAYLES: *Diamond Pet Food, Topeka, KS, USA*
BABAK GIVEHCHI: *CPReg Consultants, North York, ON, Canada, Canada*

Everyone's heard of a biofilm. These microbial communities are slimy, wet, and may contain pathogenic bacteria that contaminate food and drive foodborne illness. A misconception in the understanding of biofilms is these need high levels of moisture and enough nutrients to form and survive. Research has demonstrated this is not the case. These "dry surface biofilms" are not visible to the human eye, cannot be detected with either dry or wet swabbing techniques, are more resistant to the action of disinfectants than their wet counterparts, and yet their bacterial constituents, when disturbed, are still transferrable via direct or indirect contact with hands or cleaning tools. As a result, dry surface biofilms could play a surreptitious yet nontrivial role in infection transmission and food spoilage. In light of this, during this symposium, experts from academia, regulatory entities and industry will provide insights on the risks of, and mitigation strategies for dealing with dry surface biofilms that contain species such as *Salmonella enterica*, *Escherichia coli* or *Listeria monocytogenes* in food settings. First, we'll discuss the main biological and prevalence differences between dry and wet biofilms on environmental surfaces, and why the former should be more concerning from a foodborne illness risk mitigation perspective. Secondly, in addition to registered product labels, we'll unpack regulatory guidance involving biofilms that provide with direction to manage these microbial communities in food settings. Finally, we'll describe prevention and remediation strategies for biofilms on surfaces in low moisture environments, including their level of success and lessons learned. Attendees of this symposium will gain a new level of awareness of dry surface biofilms, their impact to food safety, and mitigation strategies that can be applied within their own facilities.

S7 Forever Chemicals: The Past, Present and Future of PFAS in Food

CHERYL MURPHY: *MSU Center for PFAS Research, East Lansing, MI, USA*
LUC PELLETIER: *Health Canada (Bureau of Chemical Safety), Ottawa, ON, Canada, Canada*
RYAN MATSUDA: *United States Department of Agriculture, Food Safety and Inspection Service, Albany, CA, USA*

Per- and poly- fluoroalkyl substances (PFAS) are a group of man-made chemicals or manufactured chemicals (i.e., non-naturally occurring chemicals) that are resistant to degradation and pose a potential threat to human health due to their bioaccumulation and persistence in the environment. Due to their amphiphilic properties, PFAS were, and still are, widely used in industrial applications and various consumer products, such as food packaging, household cleaning products, fire retardants, and wastewater treatment. As a result, water used for feed crops or live animals may be contaminated from industrial and waste-stream sources resulting in PFAS entering the food supply via contaminated meat, poultry, and fish products. Toxicological studies have shown that PFAS exposure at high levels can detrimentally affect the endocrine, cardiovascular, and immune systems, with studies also indicating that high levels of exposure can lead to newborn infant health problems. Therefore, many countries have begun to phase out the use of PFAS.

Due to health and environmental concerns, PFAS has gained worldwide attention from the scientific and regulatory communities. Many government agencies, academic institutions, and industry partners either have taken on surveillance initiatives involving PFAS contamination in food, identified toxicological effects of PFAS exposure from food, or explored the fate and impact of PFAS in the environment with relation to food.

This symposium session presents the surveillance work conducted by different government agencies, demonstrates the analytical capabilities of industry partners, and showcases the research initiatives and goals by different academic institutions to determine the future for monitoring the forever chemical, PFAS, in the food supply.

S8 Mexican Papaya Safety: A Case Study in Collaboration, Education and Root Cause Analysis

DANTE GALEAZZI: *TIPA, Mission, TX, USA*
HECTOR DEL RAZO VARGAS: *Proexport Payapa, Colima, CL, Mexico, Mexico*
SERGIO NIETO-MONTENEGRO: *Food Safety CTS, El Paso, TX, USA*
TREVOR GILBERT: *FDA, College Park, MD, USA*

Papayas from Mexico were repeatedly associated with outbreaks of *Salmonella* (8 between 2011 and 2019), and FDA testing at the border revealed a high violation rate (15.3% of 3 months' samples in 2011). This prompted a diverse group of stakeholders: Mexican papaya growers, U.S. importers, and an international collection of regulators, academics, researchers and associations on both sides of the border, to work together on a multi-year, multi-pronged effort to improve the food safety practices of papayas from Mexico. This symposium will explain how stakeholders were identified and engaged to develop science-based best practices, guidance documents, verification protocols, and how these best practices were then communicated and implemented within the industry, spanning the Mexican growers through U.S. importers, all the way to retailers. The educational strategies and associated verification efforts will be described, along with a novel industry-driven process for completing a root cause analysis. The support from regulators from Mexico and the U.S. aided this process. This effort can serve as model for collaboration that yields long term benefits to the industry and consumers.

S9 Internal Audits: Are They Underestimated as a Critical Management Tool?

NIC SHARMAN: *Nic Sharman Consultancy, Didcot, United Kingdom, United Kingdom*
JESSICA BURKE: *BRCGS, Milton, ON, Canada, Canada*
ELLEN EVANS: *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom, United Kingdom*
HELEN TAYLOR: *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, Wales, United Kingdom, United Kingdom*
ELIZABETH SANTOS: *Maple Leaf Foods, Mississauga, ON, Canada, Canada*

Internal audits are a powerful food safety tool when effectively implemented throughout the global food supply chain. The aim of this symposium is to discuss the purpose and function of internal audits from four perspectives: what the requirements are from Global Food Safety Standards; the fundamental aspects of internal audits; perceptions of auditors and auditees, and an industry case study.

The symposium will begin with an introduction on what the GFSI requirements are and how they translate into the BRCGS standard for internal audits. A representative from BRCGS will share repeat non-conformances. Leading on from this we will explain the detail and approach required to create a robust and effective internal audit system.

The second speaker will share qualitative findings from research undertaken with qualified auditors and technical leaders which explore the opportunities, motivations, and capabilities of SME food manufacturers to benefit from internal audits.

The final speaker will provide a valuable case study demonstrating best practice in relation to internal audits and the links to organisational culture. The session will discuss how Internal Audit is a foundational element of the company's foods safety journey, its origins and approach, and how coaching is used to drive people development and change.

This symposium will give attendees beneficial insight on how to support manufacturing businesses to ensure internal audits are beneficial for organisational management and as a reference point to key indicators of product safety and quality.

Cumulatively this symposium will consider how the dimensions and critical components of food safety culture relate to successful and effective internal audits in food manufacturing businesses to ensure standards are improved and there is a mindset for continuous improvement.

S10 Foodborne Listeriosis in Canada: Are We There Yet? Insights into Progress and Lessons Learned Since Our Infamous Deli-Meat Outbreak

BRENT AVERY: *Public Health Agency of Canada, Guelph, ON, Canada, Canada*
MARIE BRETON: *Health Canada, Ottawa, ON, Canada, Canada*
MICHAEL G. GAENZLE: *University of Alberta, Edmonton, AB, Canada, Canada*
CHANDRE VAN DE MERWE: *University of Alberta, Edmonton, AB, Canada, Canada*
LYNN MCMULLEN: *University of Alberta, Edmonton, AB, Canada, Canada*
SPIR MARINAKIS: *Maple Leaf Foods, Mississauga, ON, Canada, Canada*
RANDY HUFFMAN: *Maple Leaf Foods, Mississauga, ON, Canada, Canada*

Listeria monocytogenes is an important foodborne pathogen in Canada, as well as globally. This bacterium can grow at temperatures as low as 0°C and is one of the most virulent foodborne pathogens in that 20–30% of foodborne listeriosis infections in high-risk individuals are fatal. As a result, listeriosis ranks third in the total number of deaths among foodborne bacterial pathogens with case-fatality rates that exceed *Salmonella*. *L. monocytogenes* have been isolated from numerous mammalian species, birds, and in some species of shellfish and fish along with soil, silage and other agro-ecosystem environments. This bacterium is associated with different food products, such as pasteurized or raw milk, soft-ripened cheeses, ice cream, novel plant-based products, fresh produce, fermented sausages, raw or cooked poultry, smoked fish, hard-boiled eggs, etc. and is capable of surviving under unfavorable effects of drying and freezing. The 2008 Canadian listeriosis outbreak was a deadly outbreak of listeriosis which was linked to contaminated deli meats. This outbreak spurred much interest in research in Canada, as well as lead to a number of changes in the way the organism is controlled and regulated. This 3-hr symposium includes speakers working in academia, government, and food industry in Canada, who will take a look back to summarize the research, surveillance and policy activities related to *Listeria* in Canada in the past two decades, as well as look ahead to the future. The topics will include detection, surveillance, control as well as policy and regulatory interventions. The outcomes of this symposium will hopefully spur novel cutting-edge research on this pathogen in Canada and beyond, and help to develop systemic strategies to control this important foodborne pathogen in the food supply.

Symposia

S11 Genomics in Food Safety: How to Use the Tools to Prevent Outbreaks

JESSIE HEIDENREICH: *Hilmar Cheese Company, Hilmar, CA, USA*
YAN LUO: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
MARTIN WIEDMANN: *Cornell University, Ithaca, NY, USA*

The IAFNS Food Microbiology Committee is committed to proactively improving the understanding and control of microbial food safety hazards to enable scientifically informed decision making. This symposium will highlight recent advancements in genomic tools used in both the public and private sector to identify and prevent foodborne outbreaks. Topics include outbreak detection and prevention, implementation of sampling, genomic fingerprinting, and genetic analysis within the food processing environment to improve food safety and public health.

S12 Molding the Future: Best Practices and New Horizons in Modeling and Quantification of Molds

PANAGIOTIS SKANDAMIS: *Agricultural University of Athens, Athens, Greece, Greece*
EMILIA RICO: *BCN Labs, Rockford, TN, USA*
FRANK SEGERS: *Corbion, Gorinchem, Netherlands, Netherlands*

Mold spoilage causes substantial food waste worldwide. Additionally, molds can cause health effects if food is ingested when mycotoxins, produced by molds, are present. Quantification of mold proliferation and growth in foods is critical to formulate food products with extended shelf life. Molds usually contaminate food via spores that grow with hyphae in three dimensional structures. This makes quantification and predictive modelling of mold spoilage difficult, and therefore, techniques designed to predict and quantify bacterial growth, for example, are less suitable for molds. In this symposium, the application of a common microbiological modeling technique—the gamma concept, wherein intrinsic and extrinsic factors that impact growth are considered independent but can be multiplied together to determine a final growth rate—will be discussed in relation to mold prediction, including its limitations and potential opportunities. Examples from real-world data and corresponding modeling validation outcomes will be shared. The various approaches to challenge testing mold control and mycological enumeration will be presented, and the benefits of future standardization of both challenge studies and enumeration will be discussed, with potential ways forward highlighted. Finally, an industry perspective on mold control will be given. First, modeling shelf life of bread formulated with mold inhibitors will be explored. Second, the design of mold challenge tests and their shortcomings will be explained. Third, new ways of mold spoilage quantification (e.g., medium- or high-throughput image analysis and in situ thermal activity monitoring) will be assessed.

S13 Achilles' Heel: The Local and Global Ramifications of Food Safety Challenges in Low-and Middle-Income Countries

ISSMAT KASSEM: *Center for Food Safety, University of Georgia, Griffin, GA, USA*
GUMATAW ABEBE: *Department of Business & Social Sciences, Dalhousie University, Truro, NS, Canada, Canada*
JORGE PINTOFERREIRA: *FAO, Rome, Italy, Italy*

Outbreaks of foodborne illnesses adversely impact health care systems as well as national food brands, trade, tourism, and socioeconomic growth, especially in low- and middle-income countries (LMICs). In these countries, foodborne illnesses perpetuate the cycle of disease, malnutrition, and poverty, which significantly affect the most vulnerable populations and fledgling industries. Indeed, the World Bank estimated the yearly cost of treating foodborne illnesses at $15 billion and total productivity loss in LMICs at $95.2 billion. However, the ramifications of foodborne illnesses are not confined within the borders of LMICs and can spill out to affect other countries. The latter is especially pertinent when considering the globalization of food supply chains and the need to cope with an increasing human population and consumer demands. Consequently, the safety of food and/or raw material imported from LMICs can have a wide transboundary impact. Therefore, we argue that there is a global interest and an obvious public health and economic benefit in controlling foodborne diseases in LMICs. In this symposium, experts will share novel data, case studies, and efforts that shed light on the local, regional, and global ramifications of food safety in LMICs.

This symposium will strengthen the global scope of IAFP by raising awareness to the following areas:

- **Major food contaminants and challenges** in LMICs.
- **The transboundary impact** of foodborne diseases affecting LMICs.
- **Gaps in the** food safety systems of important foods commodities in LMICs.
- **The essential need for sharing expertise and support robust food safety systems and surveillance programs** to monitor and control foodborne contaminants in LMICs.

Intended Audience:

Food safety professionals, academia, government representatives, industry leaders, and students that are interested in the food safety in LMICs and the global health and economic ramifications of foodborne diseases.

S14 Food Safety Culture and HACCP – The Unification Necessary for Effective Food Safety Management

ANDREW CLARKE: *Loblaw Companies Limited, Etobicoke, ON, Canada, Canada*
SHINGAI P. NYARUGWE: *University of Central Lancashire, Preston, United Kingdom, United Kingdom*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*

There has been an exponential demand for food safety culture plans, GFSI-certified sites scrambling to maintain certification with limited expertise or knowledge in this subject matter. In addition we also see an emphasis on this important topic area within Codex HACCP and regulatory standards (e.g., EU852 and FDA's 4th Pillar). A formal assessment of food safety culture or behaviours is not included within inspections or certification auditing, just the presence of a food safety culture plan devised by the FSQA group to meet a requirement, not to change culture.

This knowledge gap as to why a food safety culture plan is an essential and intrinsic part of food safety management is overlooked during food safety audits and inspections or misunderstood. For example, when a food safety culture plan was initially added to the BRCGS audit standard, over 20% of sites received non-conformances. Severe consequences can also occur, Jensen Farms, Blue Bell and Peanut Corporation of America all maintained inspected food safety programs, but all experienced product safety recalls due to failures in program application. Solutions are available to food business owners/leaders, auditors and inspectors to ensure HACCP and food safety programs truly function and do not continue to create a false sense of security.

The symposium will provide data driven insights into the output from a program of food safety audits which include an assessment of food safety culture. Discussion will identify how this can often provide contradictions but a more insightful and comprehensive understanding of the overall effectiveness of a food safety plan when behaviours are evaluated in conjunction with programs.

Symposia

Additionally, we shall explore the behaviours of the HACCP/Food Safety lead and food safety scribe and determine through published research and real world examples how these important roles can influence food safety culture and encourage HACCP team members participation in food safety decision making or potentially discourage engagement when facilitation is ineffective.

S15 Crowdsourced Data for Foodborne Illness Outbreak Investigations: Utility and Challenges

BENJAMIN CHAPMAN: *Department of Agricultural and Human Sciences, North Carolina State University, Raleigh, NC, USA*
JENNIFER BEAL: *U.S. Food and Drug Administration, College Park, MD, USA*
LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
ANNA MANORE: *Public Health Agency of Canada, Ottawa, ON, Canada, Canada*

Crowdsourced data has been routinely used for a variety of purposes, including marketing, monitoring product quality, innovation and ideation, emergency response, and trend surveillance. A growing area of interest among food safety professionals and the public at large is how crowdsourced information can be employed for food safety purposes. Social networking sites (e.g., Twitter, Facebook, Reddit, etc.), sites focused on foodborne illness reporting (e.g., iwaspoisoned.com), and a variety of other online forums (e.g., Yelp), allow individual consumers to share details of symptoms, illnesses, and/or experiences with specific food products, companies, or brands and retail or dining locations, all of which are valuable data during an outbreak investigation. However, because these platforms are open to everyone, posts can vary widely in their format and content. These inconsistencies can make it difficult to determine the validity and accuracy of the posted information and to interpret these data sources. Furthermore, anonymous posts can make further investigation with consumers extremely challenging, if not impossible. In addition to reviewing unprompted posts on social networking sites, public health professionals may also create their own posts to widely disseminate information/early signals for potential outbreaks within their jurisdictions, or to elicit specific kinds of data from social network site users (e.g., surveys to gather data about a healthy reference population). As the world increasingly utilizes digital tools to report, monitor, and act on poor food safety practices and clinical symptoms from suspect foodborne illness events, public health professionals and food safety regulators continue to evaluate these tools to determine their usefulness in outbreak investigations. This session will explore the landscape of available crowdsourced information and discuss the advantages and disadvantages of using these tools during outbreak investigations in the United States and Canada, with speakers at the federal and state/province for each country. Foodborne illness outbreak investigators from both countries will share their experiences, both good and bad, using crowdsourced information as part of a foodborne illness investigation.

S16 Alternative Protein and Novel Foods… What Could Possibly Go Wrong? Prioritizing Food Safety in Food Tech 2.0

TODD NAPOLITANO: *Synergistics Capital Consulting, Philadelphia, PA, USA*
VIJAY KRISHNA: *Glanbia Performance Nutrition, Downers Grove, IL, USA*
ROBYN EIJLANDER: *NIZO Food Research, Ede, Netherlands, Netherlands*
YANYAN HUANG: *ADM, Longmont, CO, USA*
ROBYN EIJLANDER: *NIZO Food Research, Ede, Netherlands, Netherlands*
AARON PLEITNER: *Impossible Foods, San Francisco, CA, USA*

Food Tech 2.0 is here and with it revolutionary advances in what, why, and how food is created and consumed. Traditional boundaries are being blurred everywhere… food and science, food as science, food as supplement, supplement as food, health, wellness, sustainability, transparency. The benefits are often emphasized, but are the risks as clearly assessed and elaborated? For that matter, to what extent do we even understand the inherent and unique challenges at the border between Food Tech 2.0 and food safety?

As much as Food Tech 2.0 pushes the limits of innovation in the name of sustainability, it also pushes the limits of traditional food safety. For example, how do we create mitigation strategies for novel foods we don't yet understand or design and assess food safety protocols if we don't fully comprehend the risks? How do we build predictive risk models when we are just beginning to understand the nature of the risk itself?

Perhaps, then, this is also the time for *Food Safety 2.0*, a production environment where *safety is the price of entry* for alternative and novel products and ingredients. How can we promote innovation towards a more sustainable future *while also* ensuring consumer health and safety? Given where we are now and where we are headed, we will delve into the convoluted world of alternative proteins, novel foods, and synthetic biology concluding with a regulatory overview and the question, what does the audit of the future look like?

In conjunction with the Alternative Protein and Novel Foods PDG, this three-hour symposium will explore the current landscape of Food Tech 2.0 from the inside. Individual presenters will address the following issues allowing time for Q&A at program conclusion.

S17 Under the Weather: Influence of Weather Conditions on Produce Safety

MARIA BRANDL: *Produce Safety and Microbiology Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Albany, CA, USA*
GOVINDARAJ DEV KUMAR: *University of Georgia, Griffin, GA, USA*
SHIRLEY MICALLEF: *Center for Food Safety and Security Systems, College Park, MD, USA*

Enteric pathogens can enter the food chain from environmental matrices such as soil, irrigation water and dust. Studies have shown that foodborne bacterial pathogens such as *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* can survive in soil and irrigation water for extended durations until they come in contact with a host. Produce can be contaminated by these pathogens in the pre-harvest environment. Pre-harvest contamination of produce is often insidious as it results in cross-contamination of uncontaminated batches during post-harvest handling steps such as washing, comminution and packing.

The contribution of weather conditions in the survival and cross-transfer of foodborne pathogens is understudied. Anecdotal evidences about events that lead to contamination of produce often allude to weather or climatic phenomena such as drought, heat, humidity and wind gust.

The weather can fluctuate dramatically over a short duration of time and contribute to conditions that might promote food safety hazards. These include changes to the environment or alterations to bacteria or their hosts. Under inclement weather conditions environmental matrices much as water and soil can serve as vehicles for foodborne pathogen cross transfer. Rainfall, sunlight, humidity and wind speed can result in cross-contamination of produce surfaces through splashing, desiccation, condensation, and dust dispersal.

Further, foodborne pathogen populations in environmental matrices can fluctuate depending on the temperature, moisture, and UV radiation. Research has indicated that exposure of bacteria to desiccation, solar radiation, heat or frost can result in physiological alterations such as the loss of culturability, filamentation, sporulation and sub-lethal injury. Evaluating environmental conditions that might result in elevated produce contamination risks is critical for pre-risk management.

The symposium is being proposed to discuss findings about the roles of weather and climate associated environmental stressors on bacterial persistence, survival and cross transfer to produce. The evaluation of food safety risks associated with climate and weather change can help fortify pre-harvest food safety. Understanding the effect of weather on the pathogen, produce and the environment can help in improved risk assessment.

S18 Human Enteric Viruses, A Risk Analysis Approach for the Soft Fruit Industry

LEE-ANN JAYKUS: *Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC, USA*
DONALD W. SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
SANJAY GUMMALLA: *American Frozen Food Institute, Bethesda, MD, USA*

The potential for soft fruits to serve as vehicles of infection with human enteric viruses is now well accepted. The industry seeks to manage potential Hepatitis A virus (HAV) and human Norovirus (hNoV) contamination to fresh and frozen products. This group of non-cultivable human pathogens presents unique challenges including how and where contamination occurs; how contamination might be prevented and/or controlled; the nuances associated with testing along the supply chain; and interpretation of results from the standpoint of public health risk. This symposium will provide a summary of virus-berry outbreak trends over the last two decades, key features that contribute to the ability of hNoV and HAV to persist in the environment, resist inactivation, and ultimately infect humans will be outlined with an eye to capitalizing on this information for prevention and control purposes. The application of a risk analysis approach for addressing HAV and hNoV in soft fruit will be discussed by speakers walking through all elements, risk assessment, risk management, and risk communication. Attendees will get a feel for how science-based evidence should be considered for effective policy development concerning viruses, what strategies risk managers are using to stay nimble in this challenging environment, and the vital role effective risk communication can play for quick industry uptake.

S19 Current Options in Evaluating the Infectivity of Human Noroviruses and Their Potential Application in Food Safety

JAN VINJÉ: *Centers for Disease Control and Prevention, Atlanta, GA, USA*
DAN LI: *National University of Singapore, Singapore, Singapore, Singapore*
ALVIN LEE: *Institute for Food Safety and Health, Bedford Park, IL, USA*

Foodborne viruses represent a serious threat to human health, with human noroviruses accounting for the vast major of viral food outbreaks. Most foodborne viruses in the environment are resistant to environmental conditions, several disinfectants, and many food manufacturing treatments. Detection and quantification of foodborne viruses is mainly performed by molecular methods however evaluation of virus infectivity is challenging.

In the absence of a robust in vitro cell culture system for human norovirus, inactivation studies have been performed by conducting clinical trial studies. To overcome ethical issues and generate a large amount of dataset, a lot of research relies on surrogates, such as murine norovirus, feline calicivirus, and Tulane virus and MS2 phage. Alternatively, approaches based on capsid integrity coupled with quantitative reverse transcription real-time PCR (RT-qPCR) have been reported, including porcine gastric mucine (PGM) assays to selectively bind infectious human norovirus. Recently, a novel three-dimensional (3D) cell culture technique based on non-transformed stem cell-derived human intestinal enteroids (HIEs), which recapitulate the complexity and cell diversity of the gastrointestinal tract has been reported to be permissive towards human norovirus infection. In parallel, it has been found that zebrafish larva also allows the replication of human noroviruses. However, both approaches are still at the dawn of its application in food and environmental virology field. In this symposium, we will discuss the use of the currently available methods to assess norovirus infectivity and its application to improve food safety.

S20 Testing and Improving HACCP Team Proficiency to Strengthen Food Safety Culture

CAROL WALLACE: *University of Central Lancashire, Preston, Lancashire, United Kingdom, United Kingdom*
LONE JESPERSEN: *Cultivate Food Safety, Hauterive, Switzerland, Switzerland*
JOHN PETIE: *MidWestern Pet Foods, Evansville, IN, USA*

For years, food companies have invested in collecting food safety data which are typically used to demonstrate how compliant HACCP programs are to external requirements and more recently to measure where their food safety cultures are at on a culture maturity continuum. These measures are often calculated based on data collected through surveys and number of non-conformances against a company's HACCP program. Following data gathering, decisions are made for how to be compliant next time an inspection or audit comes around but, after this, the data are often forgotten, hidden away in filing systems, providing no further value to the company. This information wastage goes against the philosophy of HACCP as a preventative continuous improvement approach to food safety and reduces HACCP to a paperwork exercise. HACCP data and review activities provide learning opportunities for companies to modernise risk management and overcome the complacency often associated with ageing HACCP plans. This requires understanding of HACCP Team dynamics and HACCP proficiency to maximise HACCP program upgrades and use them as a basis for strengthening food safety culture.

Participants will hear about factors influencing HACCP effectiveness, including the influence of human factors. Using a case-study of a North American pet food company, data were collected at three manufacturing locations for food safety culture and HACCP proficiency. Results were tested using ANOVA and found to be significantly different before and after intervention for all three plants. The proficiency levels improved on average 49% post-intervention and the significant factors causing this improvement were identified through factor analysis. Speakers will share results from this case study and other global studies of HACCP teams, HACCP team interventions, and actions required from a practitioner perspective to make lasting and significant changes. This will include correlation of HACCP team and individual team member proficiency to food safety culture and discussion of practice methods and tools that leaders have used to make decisions and change behaviours and improve food safety management and culture.

S21 Understanding Cell-Cultured Seafood and Its Food Safety Challenges

REZA OVISSIPOUR: *Virginia Tech, Hampton, VA, USA*
MARTIN DUPLESSIS: *Food Directorate, Health Canada, Ottawa, ON, Canada, Canada*
NOREEN HOBAYAN: *BlueNalu, San Diego, CA, USA*

Although symposia about cell-cultured (cultivated) foods have been presented at previous IAFP annual meetings, none have focused on seafood. Unlike beef, pork, dairy, poultry and eggs, hundreds of aquatic animal species are consumed by humans. The diversity of seafood (finfish, crustaceans, mollusks) along with environments in which they grow (wild vs farmed; salt vs brackish or freshwater) and manner in which they are consumed (raw, partially or fully cooked, reheated) necessitate a stand-alone session on cultivated seafood products. Current and soon-to-be cultivated seafood include tuna, salmon, snapper, mahi-mahi, oysters, crab, shrimp, lobster and beluga caviar.

According to the Good Food Institute's database, 24 companies headquartered in the following countries are developing cultured seafood products: Australia, Canada, China, Estonia, France, Germany, Israel, Japan, Latvia, Netherlands, Russia, Singapore, South Africa, South Korea, Switzerland and U.S.

(California, North Carolina, Washington, Wisconsin). However, as of October 2022, Singapore is the only country that has approved sale of cultivated meat (in November 2020) with chicken being the first product commercialized. Another company plans to build a Singapore facility to produce cultivated shrimp. Advantages of cultivated seafood include: reduces pressure on wild stocks; more humane and sustainable; not constrained by seasons, weather conditions or fishing limits; consistent supply; easier to trace; minimizes species substitution (fraud); less waste; and longer shelf life.

While companies developing cultivated seafood claim it is free from conventional seafood safety hazards such as environmental contaminants, natural toxins, parasites, microplastics, aquaculture drugs, antibiotics and enteric pathogens that plague wild and/or farmed seafood, is that true? Are new or unanticipated hazards created or introduced in the production of cultivated seafood? Join us for this symposium to learn how seafood is cultivated, how it is regulated, and how its safety is controlled.

S22 Control of *Cronobacter* and *Salmonella* in Low-Moisture RTE Facilities Using Dairy Examples

DAVID COOK: *Commercial Quality & Food Safety Solutions, Inc., Richmond, IL, USA*
MONTGOMERY BOHANAN: *Leprino Foods, Denver, CO, USA*
KAREN MCCARTY: *Agropur, Inc., Le Sueur, MN, USA*

This session will focus on existing best practices and enhancements needed for effective control of *Cronobacter* and *Salmonella* in low-moisture RTE facilities using dairy examples. The speakers are expert practitioners from the industry who are responsible for protecting products and consumers every day. We will briefly cover many aspects of pathogen controls with deeper dives into three topics. The proposed talks are *Cronobacter* & *Salmonella*: 1) Facility and Equipment Design Considerations, 2) Cleaning and Sanitation in a Dry RTE Environment, and 3) Pathogen Control Programs & Enhanced Environmental Monitoring

The session is being convened by The Innovation Center for U.S. Dairy which provides a collaboration forum for processors to work together pre-competitively on various aspects of Food Safety. Since 2014 we have had focused groups of experts sharing on low moisture dairy powder pathogen controls best practices resulting in a two-day workshop, the guidance document Control of Pathogens: Guidance for the U.S. Dairy Industry, webinars, and additional tools designed to help processors of all sizes.

S23 Serogroup Independent Detection and Isolation of Shiga-Toxin Producing *E. coli* – Are We Really Ready for This?

ROBERT BARLOW: *CSIRO, Brisbane, Australia, Australia*
MICHAEL DAY: *USDA-FSIS, Athens, GA, USA*
JAMES BONO: *USDA, ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*
JOSEPH BOSILEVAC: *U.S. Department of Agriculture – ARS, U.S. Meat Animal Research Center, Clay Center, NE, USA*

Shiga-toxin producing *E. coli* (STEC) are the cause of numerous illnesses, outbreaks, and recalls each year. STEC serogroups O26, O45, O103, O111, O121, O145, and O157 have been identified as the cause of most STEC infections around the globe. Thus, they are often regulated or monitored in some fashion. However, additional serogroups such as O104 and O80 have emerged in the EU as problem strains. Because of this, recent expert panels and agencies (NACMCF, FAO, FSIS, EFSA) have proposed that it would be prudent to monitor food safety systems for any STEC capable of posing a public health risk regardless of its serogroup or serotype. Yet, test methods continue to rely on serogroups in one way or another to detect or isolate STEC. If we are to move towards detecting and isolating any disease causing STEC, alternate gene targets (virulence factors) specific to STEC will be needed to improve the reliability of the detection assays. Further, culture isolation for STEC will need to take a leap forward as well if O group specific isolation methods are to be left behind. In this session, the background and impetus for moving away from the current STEC serogroups will be presented with thoughts on how the redefinition will impact industry, markets, and public health. Then the latest research on approaches to detect STEC using conserved virulence factors, and to isolate STEC using improved reagents will be described.

S24 Diversity, Equity, Inclusion, and Belonging Considerations across the Food Supply Chain

VIJAY KRISHNA: *Glanbia Performance Nutrition, Downers Grove, IL, USA*
SUZANNE HATHAWAY: *Maple Leaf Foods, Mississauga, ON, Canada, Canada*
TAMIKA SIMS: *IFIC, Washington, DC, USA*

Diversity, Equity, Inclusion, and Belonging (DEIB) efforts within organizations often center themselves on embracing the differences—such as race and ethnicity, cultural heritage, sexual identity and/or orientation, and differing abilities—of the individuals within their organizations and teams. In addition, these efforts shine a light on how differences can influence how each person thrives within an organization, revealing what specific working conditions may help them feel most supported by their colleagues and underscoring how individuals do not need to overly assimilate to be considered an integral and respected part of their team. Likewise, successful DEIB efforts do not seek to embrace blanket statements such as "We are all the same"—rather, they highlight that we all have differences (some visible, some not visible), and that these differences do not make any one person better, more productive, or more valued than another.

As diversity in our society evolves, so does the composition of food industry stakeholders, and enhanced understanding of diversity should be considered mandatory for the food industry to remain at the forefront of modernized productivity. An intentional focus on DEIB efforts is needed among all stakeholders, including regulatory agencies, academic institutions, and well-established organizations such as IAFP.

This symposium will feature an overview of IAFP's progress to date on its two-year DEIB journey. In addition to IAFP's progress to date, it will explore how various sectors of the food production and food safety industries have worked with their teams to integrate DEIB practices into their teams' daily practices both internally and externally. This will include a discussion about challenges, opportunities and practical examples that have been utilized within individual organizations to render positive outcomes.

S25 Outbreak Symposium

NICOLE STONE: *Indiana Department of Health, Indianapolis, IN, USA*
SHARON SEELMAN: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, Coordinated Outbreak Response and Evaluation Network, College Park, MD, USA*
LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
EWEN TODD: *Ewen Todd Consulting LLC, Okemos, MI, USA*
JENNIFER FREIMAN: *USDA-FSIS-OPHS, Washington, DC, USA*
EVELYN PEREIRA: *U.S. Food and Drug Administration – CFSAN, Coordinated Outbreak Response and Evaluation Network, College Park, MD, USA*
THAI-AN NGUYEN: *U.S. Centers for Disease Control and Prevention, Atlanta, GA, USA*
HEATHER BOND: *Public Health Agency of Canada, Guelph, ON, Canada, Canada*
AARON BECZKIEWICZ: *USDA-FSIS, Washington, DC, USA*
LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
JENNIFER FREIMAN: *USDA-FSIS-OPHS, Washington, DC, USA*
KARI IRVIN: *U.S. Food and Drug Administration, College Park, MD, 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. CDC and FDA will discuss 3 outbreaks of *Salmonella* Typhimurium and *Salmonella* Newport infections in 2012, 2020, and 2022 that were linked to cantaloupe and watermelons grown in the same region of Indiana. Speakers will describe an enhanced surveillance project focused on these *Salmonella* strains, laboratory findings from environmental sampling and prevention activities. An overview of several international outbreaks will be provided by Dr. Ewen Todd. Outbreaks covered in the review include outbreaks covered in the review include microsporidium Enterocytozoon from a Danish workplace canteen; *Salmonella* Welikade from goat cheese in France; *Cryptosporidium* from romaine in Sweden; botulism from homemade food in Italy. In 2022, there was an outbreak of *Salmonella* infections and an outbreak of *E. coli* O157:H7 infections linked to two separate restaurant chains. Representatives from CDC, FDA/USDA, and industry will discuss the epidemiology, traceback, and public communications challenges surrounding multistate outbreaks linked to restaurants. An FSIS speaker will discuss a *Salmonella* Typhimurium outbreak linked to salami sticks investigation, USDA research priorities that came from this outbreak. A speaker from the Public Health Agency of Canada will review a 2022 multi-provincial outbreak of norovirus and acute gastrointestinal illness. A total of 60 cases from four provinces were investigated, and consumption of raw and cooked spot prawns was identified as the source of illness. Although root cause could not be determined, hypotheses include contamination of the harvest areas by sewage outfalls, contamination in water used to fill live tanks, and contamination at the processor. This was the first multi-provincial outbreak of norovirus associated with spot prawns in Canada.

S26 Controlled Environment Agriculture (Hydroponic/Aquaponic) Research Updates

JENNIFER PERRY: *University of Maine, Orono, ME, USA*
SANJA ILIC: *The Ohio State University, Columbus, OH, USA*
SOCRATES TRUJILLO: *U.S. Food and Drug Administration, College Park, MD, USA*

Interest in Controlled Environment Agriculture (CEA), such as hydroponics (HP) and aquaponics (AP), has steadily grown over the last decades. CEA offers advantages over traditional, soil-based agriculture since it offers protection from the environment, better water and nutrient use, and year-round growing. It also negates the need for arable land and can be leveraged as use for vacant existing properties. When growing produce in soilless conditions, the microbiota to which produce is exposed differs significantly with regard to composition and uniformity from that expected in soil-based systems. However, the drivers of these microbiota, and the level of risk associated with incidental foodborne pathogen exposure, are not thoroughly understood. The need for a more holistic understanding of CEA systems is highlighted by recent outbreaks implicating hydroponic produce. In this symposium, researchers from academic institutions and government bodies will summarize new research on this topic and discuss continuing needs for further research in this area.

S27 Sustainability: Is Food Safety Compromised as a Byproduct?

ANGELA ANANDAPPA: *Alliance for Advancing Sanitation and Northeastern University, Glenview, IL, USA*
JEFFREY LEJEUNE: *FAO, Rome, Italy, Italy*
CARI RASMUSSEN: *Commercial Food Sanitation, Springfield, MA, USA*

What food safety concessions have we made in the pursuit of sustainability? Sustainability has been a leading initiative of the food industry for years. As the concept continues to evolve, regulations and independent certifications have slowly incorporated these ideals. The food industry has implemented concepts of sustainability from chemical selection, water use reduction and bio-friendly packaging based on consumer and environmental demands. The question becomes, what are the unintended consequences that result from these actions? In this symposium, global experts from industry and FAO will discuss these latent outcomes by address the most pressing questions. Can food safety hazards be incurred using these practices? Is food safety compromised as a by-product of the sustainability efforts? Does the reduction of water in cleaning practices reduce efficacy? What approaches in application and chemistry selection minimize these potential outcomes? Listeners of the symposium will walk away with a deeper understanding of the second and third order effects of their organizations sustainability goals, There is no doubt sustainability is a necessary method to ensure future generations have the resources to meet their needs but the cost to the production of safe, wholesome food cannot be compromised through these measures.

S28 Challenges and Opportunities Navigating Requirements of Ready-to-Eat and Not Ready-to-Eat for Refrigerated and Frozen Foods

SANJAY GUMMALLA: *American Frozen Food Institute, Bethesda, MD, USA*
TBD TBD: *TBD, TBD, AL, USA*
OBADINA ADEWALE: *Federal University of Agriculture, Abeokuta, Nigeria, Nigeria*

Significant innovation in the refrigerated and frozen areas of the grocery store in the past decade has enabled a wide variety of choice to the consumer. New ingredients, processes, packaging technologies, and even product categories have provided today's consumer an array of choice. Despite this innovation, or because of it, food processors are faced with a multitude of challenges in providing consumers with products that balance food safety with sensory and quality attributes over the product shelf life. Producers of ready-to-eat (RTE) foods must ensure their products are formulated, processed, packaged, and stored such that, they are intended for direct consumption without additional cooking steps or processes to eliminate or reduce pathogens

of concern. Not-RTE (NRTE) foods require validated preparation instructions on the label to instruct consumers how to prepare the food to ensure that it is safe for consumption. However, adulterating microorganisms are still not permitted in such products. Moreover, there is increasing regulatory and end customer pressure to tighten microbiological requirements on NRTE products due to concern about consumer deviation from the printed preparation instructions. RTE foods have been associated with illnesses and outbreaks resulting from poor sanitation, inadequate processing, abuse during storage, or modified exposure before consumption that may support growth of pathogens to high levels. Steps to address food safety concerns such as appropriate GMPs, processing parameters, and consumer education supported by science-based and risk-based regulatory approaches help mitigate risks associated with the prevalence of pathogens. This symposium will explore the challenges and opportunities across the global supply chain in product development, processing, regulatory compliance and consumer behaviors that blur the distinction and food safety expectations between these foods.

S29 How Wet is Wet Enough? The Importance of Proper Hydration in Thermal Processing of Aseptic and ESL Refrigerated Beverages

ROBYN EIJLANDER: *NIZO Food Research, Ede, Netherlands, Netherlands*
ANDRÉ REHKOPF: *Saputo, Sacramento, CA, USA*
ROBERT W. MANNING: *Niagara Bottling, Diamond Bar, CA, USA*

Commercial losses and recalls due to spoilage of aseptically packaged shelf-stable and extended-shelf-life (ESL) refrigerated beverages, while still relative rare, are increasing in frequency due to a variety of reasons. These include new and inexperienced entries to the segment, longer production runs, use of new and exotic plant-based ingredients, difficulty in hydrating hydrophobic ingredients and high bacterial spore loads in incoming ingredients. While rare, once a spoilage event occurs in this category, the 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 lack of knowledge on the correlation between levels, types and properties of bacterial spores. These challenges call for holistic and multidisciplinary contributions to provide for root cause-analysis and prevention of spoilage issues.

A common cause of spoilage in this category is the improper hydration of hydrophobic ingredients such as cocoa or high protein powders. This situation is often misdiagnosed and may result in sporadic, low-level spoilage that may reoccur for weeks or months and in some cases have resulted in the closing of whole production lines. The objective of this symposium is to provide insight in procedures and strategies to investigate and prevent spoilage issues in this category with strong emphasis to the issue of ingredient hydration as a cause of spoilage. Presenters will illustrate the various processes and technologies available to properly hydrate key ingredients as well as provide relevant case-studies illustrating real world spoilage issues including investigational and diagnostic tools used to asses these spoilage problems.

S30 Applications of Artificial Intelligence and Machine Learning in Food Safety: An Update and Future Trends

BARINDERJIT SINGH: *I. K. Gujral Punjab Technical University, Kapurthala, India, India*
ABHINAV MISHRA: *University of Georgia, Athens, GA, USA*
CLAIRE ZOELLNER: *iFoodDS, Seattle, WA, USA*

Food safety is essential for our health and survival. Because of the diversity and intricacy of today's food production systems is immense, the highest food safety standards must be enforced through cutting-edge technologies to protect consumers from foodborne illnesses in today's complex and sophisticated food systems. The food industry incurred millions of dollars in damages due to recall costs and reputational damage. Recent advances in the data science and machine learning have resulted in the discussion of Big Data, a term that has not been traditionally associated with food safety. Food and Drug Administration (2020) announced the "New Era of Smarter Food Safety Blueprint," which outlines achievable goals to enhance traceability, improve predictive analytics, respond more rapidly to outbreaks, address new business models, reduce contamination of food, and foster the development of stronger food safety cultures by using artificial intelligence and machine learning. Artificial intelligence (AI) empowered proactive data-driven strategies can help to enhance the food safety management system in the food supply chain by alarming the food safety issues before they become serious. The discussion will focus on food safety delivery from the farm to the fork which requires a strong food safety culture, reliable data, robust database management, and technical expertise and corresponding AI system design and methodology. The overall goal is to capitalize on opportunities to leverage AI solutions that focus on consolidating food safety to find new applications from existing algorithms. These innovations can minimize food waste and significantly ameliorate sustainability.

S31 Food Safety within the Horticultural Sector in Africa

ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture, Abeokuta, Abeokuta, Ogun State, Nigeria, Nigeria*
GENET GEBREMEDHIN: *Global Alliance for Improved Nutrition (GAIN), Addis Ababa, Ethiopia, Ethiopia*
GLORIA LADJEH ESSILFIE: *University of Ghana, Legon, Ghana, Ghana*

At the 2022 IAFP Annual Meeting, a group of interested food safety experts from different regions in Africa held a short symposium on Food Safety within the context of Food Security in Africa and discussed the trade-off between food safety and security that exist in safety of food traded at formal and informal markets.

The current proposal for a short symposium follows up from that session and zooms in on those food safety challenges that are particular for horticultural products coming into food value chains and onto informal and formal markets in Africa as identified during the ACAFP Conference in 2022. Since contamination with pathogenic microorganisms and chemicals 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 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 interventions.

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 address this in the local context

S32 Aquaculture and Aquaponics: Waste Not, Want Not

JANELLE HAGER: *Kentucky State University, Frankfort, KY, USA*
JENNIFER BANACH: *Wageningen Food Safety Research, Wageningen University & Research, Wageningen, Netherlands, Netherlands*
ANDREA TARNECKI: *Auburn Universtiy, Dauphin Island, AL, USA*

Seafood aquaculture continues to expand world-wide, with an estimated 50% of seafood produced for human consumption being cultured, globally. In addition to seafood, seaweed is a growing commodity cultured for consumption. Often the types of culture systems or handling practices employed to ensure a profitable crop can unintentionally introduce potential food safety hazards. Increasingly, fish and shellfish are being cultured in or aquaponic systems. In aquaponic systems, the natural by-products of fish are used as nutrients for plants, such as lettuce and basil, being co-cultured in the system. As beneficial as these systems are for produce (and seafood) production, they inherently present numerous challenges to food safety and quality of both product types.

This session will provide an overview on both aquaculture and aquaponics systems, with a focus on potential, and identified, public health concerns. Specific examples of challenges for food safety and quality in aquaculture and aquaponic systems will be discussed.

Symposia

S33 *Campylobacter*-Associated Food Safety

XIAONAN LU: *McGill University, Sainte-Anne-de-Bellevue, QC, Canada, Canada*
QIJING ZHANG: *Iowa State University, Ames, IA, USA*
THOMAS ALTER: *Freie Universitat Berlin, Berlin, Germany, Germany*

Campylobacter is the most common laboratory-confirmed cause of bacterial foodborne gastroenteritis worldwide, accounting for 400-500 million cases of diarrhea annually. Campylobacteriosis is the most frequently reported foodborne illness in North America, outnumbering reported cases of *Listeria*, *Salmonella*, and *Escherichia coli* infections combined. According to the European Union One Health 2018 Zoonoses Report, campylobacteriosis remains the most reported zoonotic infection in humans in Europe since 2005, representing almost 70% of all reported cases. While *Campylobacter* typically causes self-limiting gastroenteritis, it can also lead to life-threatening post-infection complications, such as Guillain-Barré syndrome and septicemia. The high infection rates of *campylobacter* pose great economic burdens.

Campylobacter-associated food safety is a significant concern worldwide. Even though *Campylobacter* spp. are quite susceptible to environmental or technological stressors, they are able to survive in the environment and in food products. Both domesticated and wild birds are natural reservoirs of *Campylobacter*, and once this microaerobic bacterium colonizes the avian gastrointestinal tract, there is no effective intervention to reduce its footprint in the agro-ecosystem. Although this microbe is microaerobic, it is highly prevalent everywhere in the aerobic food processing environment, such as poultry farms, dairy farms, and animal slaughter facilities. It is not clear at what step in the food chain the cross-contamination starts to occur. Furthermore, *Campylobacter* genomes are highly dynamic thus requiring detection and intervention strategies to be adaptable to these genetic changes. Taken together, novel research works are required to better understand this microbe and its interaction with the agri-food system.

This symposium focuses on the critical perspectives of *Campylobacter*-associated food safety, such as cultivation, detection, typing, sequencing, survival, ecology, adaptation, antimicrobial resistance, and control strategy. Eventually, we aim to help the agri-food industry to develop systemic strategies to combat this microbe in the agro-ecosystem so as to improve food safety.

S34 From Inspection to Insight: Using Regulatory Retail Inspection Data to Improve Food Safety Policies and Practices

ADAM KRAMER: *Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*
BARBARA KOWALCYK: *The Ohio State University, Center for Foodborne Illness Research and Prevention, Translational Data Analytics Institute, Columbus, OH, USA*
CARRIE RIGDON: *Association of Food and Drug Officials (AFDO), Saint Paul, MN, USA*

Hundreds of thousands of retail food inspections take place every year by state and local public health agencies, generating huge amounts of data about food safety risks present at retail. Often data generated from these inspections is evaluated for one establishment or even one inspection at a time by local authorities. But advanced data analytic methods and powerful data analytics tools can help us identify effective food safety policies and opportunities for better protecting public health. Academic and industry partners can play a crucial role in collaborating with local and state public health organizations to provide analytical support and research perspective to these analyses. Recently, groups across academic, regulatory and industry, have turned to data to inform food safety decisions and policymaking. The utility of this data analysis for informing decision makers lies in the increased capacity of local and state public health organizations to collect and manage their data in a way that allows for additional analyses, appropriate application of statistical and data analytic methods that can accommodate the correlated and sometimes messy nature of inspection data, and team members that can draw meaningful insight from the results of such analyses to inform their programs. Additionally, aggregation and analysis across several individual data sets can elucidate the characteristics, environmental factors, and practices in retail and food service establishments that affect the prevalence of foodborne illness risk factors. In this symposium, researchers from across government and academia will share work that has been done recently using inspection data and discuss how powerful data analytics tools can help us see where we are making a difference and where there are still gaps in meeting our food safety and public health protection goals.

This symposium is being submitted as a companion to:

- *Practical & Effective Approaches and Uses of Data in Retail & Foodservice Food Safety Programs (roundtable)*

S35 Wait, a Sanitizer is What Now?! Paradigm Shifts in Sanitizer Regulations and How They Impact Food Safety and Sanitation Application

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If you have not already heard, the U.S. Environmental Protection Agency (EPA) is changing sanitizer regulations. Sanitizers, as you know them, only reduce bacteria. A disinfectant is required if you want a higher efficacy or a broader range, i.e., viruses. This will not be the case moving forward. The EPA plans to allow sanitizers to have virus claims without being a disinfectant. This change improves sanitation access, lowers costs to end users, and reduces pesticide overuse. But, this regulatory paradigm shift upends years' worth of education, training, and food safety regulation. So, how does this impact food safety? How do these changes impact sanitation standard operating procedures? How should I now evaluate my current sanitation portfolio? What viruses

should be controlled by my sanitizer? When is a disinfectant required now? How will the FDA Model Food Code adapt to reflect these statutory changes? Expert speakers from EPA, FDA, and academia will tackle these pressing questions. They will share regulatory updates, perspective, pain-points, and impacts to the industry. Symposium attendees will leave with a firm understanding of this new regulation, its impact to their sectors and businesses, and better understanding of utilization of antimicrobial products within food settings.

S36 Establishing Microbiological Performance Standards for Food Safety

STEPHANIE NGUYEN: *Conagra Brands, Omaha, NE, USA*
RICO SUHALIM: *PepsiCo, Plano, TX, USA*
AARON UESUGI: *Mondelez International, Columbia, MD, USA*

The Food Safety and Modernization Act's (FSMA) Current Good Manufacturing Practice and Hazard Analysis and Risk-based Preventive Controls for Human Food regulation requires that process controls for food are validated. Per the regulation, validation "must include obtaining and evaluating scientific and technical evidence (or, when such evidence is not available or is inadequate, conducting studies) to determine whether the preventive controls, when properly implemented, will effectively control the hazards." Validations for thermal processes are conducted to demonstrate that a specific thermal process can significantly minimize or prevent the pathogen of concern to a reasonably safe level.

Performance standards are the specific pathogen reduction levels that must be attained during processing to assure proper food safety has been achieved. In some cases, these limits have been established over decades of scientific testing. More often than not, these performance standards can vary depending on the food category.

Performance standards can be established based on currently available scientific literature, scientific studies performed by the company, regulatory requirements, or by using risk-based pathogen modeling.

This symposium will explore accepted industry performance standards across categories such as meats and poultry, salty snacks, juices, carbonated beverages, bakery products, and shelf-stable canned foods. For many products, specific guidance for a performance standard is not available because adequate data is not available (for all products) to establish the log reduction necessary to protect public health. In such cases, there is a need for multiple-hurdle concept that leverages scientific literature and laboratory testing data to determine appropriate performance standards for those product categories. In this symposium, experts will share practical examples and considerations for their choices (including the use of mathematical modeling in performance standards development), and verification and validation options for these types of preventive controls. It is hoped that the examples shared will spur discussions with attendees to collectively benchmark current practices among participants.

S37 When the Material Isn't Foreign: Identification and Mitigating the Risk of Inherent Physical Safety Hazards

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KEITH RHOADES: *Intertek, Arlington Heights, IL, USA*
SARAH SMITH-SIMPSON: *Gerber, Fremont, MI, USA*

The behaviors in which we consume food have dramatically changed from the inception of mandatory HACCP filings. Food and food packaging is ever evolving to meet new geographic perceptions of health, wellness, and sustainability. In the midst of these global changes, physical safety incidents are more prevalent than ever. Just in the U.S. alone, there are approximately 5,000 choking deaths per year and choking is the fourth leading cause of unintended injury death. Additionally, pets are not immune to food related safety incidents. Choking and suffocation events occur frequently, costing dog and cat owners exorbitant fees in emergency veterinary care.

When foreign material is present in food, actionable steps are relatively clear, but when the hazard is part of the intended food itself, the lines begin to blur. There is foundational knowledge that can be operationalized to reduce choking and other intrinsic physical safety risks for both humans and pets. Utilizing design hazard analysis techniques can determine whether the physical attributes of a product or package have the potential to create a hazardous condition for the consumer.

This symposium will discuss:

- Foundational knowledge around physical hazards and risks thereof.
- How to identify, assess, eliminate and/or mitigate these risks using design-based proactive interventions with special focus on vulnerable populations and identifying foreseeable use.
- Analytical evaluations to measure the severity of hazards and how physical attributes of food and food packaging may contribute to undesired outcomes.
- How and when to communicate risk to the consumer when a hazard cannot be eliminated.
- Case studies illustrating how changes to product or package design impact physical safety risk.

Takeaways will include differences in approach to demographic and geographic perceptions, how to operationalize at scale, incorporation into organizational workflow, and why cross-functional teams should collaborate to manage physical safety risks.

S38 Pressing Food Safety Issues in Some Developing Countries: Challenges and Current Trends

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KIZITO NISHIMWE: *Department of Food Science and Technology, University of Rwanda, Kigali, Rwanda, Rwanda*
JUAN SILVA: *Mississippi State University, Mississippi State, MS, USA*

There is a growing global paradigm shift in developed countries from largely implementing food safety actions at their ports-of-entry to preventive, risk-based interventions using the most current scientific information and best practices. In this regard, this symposium will focus on current practices that compromise food safety during growing, harvesting, processing, transport, storage, and distribution of selected food products in some developing countries. This symposium will also provide information on areas of improvement needed for relevant authorities and systems in those countries to effectively and efficiently regulate food safety. Although food safety as a whole is important, there are certain pressing issues that compromise production of safe food in certain parts of the world. Those issues can vary among countries globally. Therefore, this symposium will highlight important contributory factors that increase food safety risks in Rwanda, Guyana and Mexico. This symposium will shed light on food safety concerns associated with both the flow of food from farm-to-consumer and food safety regulatory deficits in those countries. Moreover, it will provide country-specific information to offer food safety scientists and allied researchers a solid factual basis for collaborative research on international food safety issues. Presenters will highlight farm-to-consumer food safety issues, and current regulatory actions unique to their respective country. They will also discuss how these issues

are presently being addressed in relation to selected food products currently being exported or with potential for export. Attendees will gain a better understanding of these issues and possibly gain a solid informational basis for establishing research linkages with institutions and relevant personnel in developing countries. While many symposia have focused on international issues, this symposium is unique in that it aims to address highly relevant, up-to-date food safety concerns in several developing counties within one symposium.

S39 What's Cooking? Lethality Processes for Scientific Gaps in FSIS' Appendix A

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SHELDON HANNA: *Smithfield, Smithfield, VA, USA*
CHRIS YOUNG: *American Association of Meat Processors, Elizabethtown, PA, USA*

In December 2021, the Food Safety and Inspection Service (FSIS) announced the availability of an updated Cooking Guideline (Appendix A). FSIS develops guidance to apply to as many products as possible; however, because processes vary widely across industry, FSIS guidance may not always be directly applicable. As part of this update, FSIS also identified several "scientific gaps" – common cooking processes for which establishments have used Appendix A as support, even though the processes cannot achieve the critical operating parameters included in the revised guideline.

In the interest of continuing to refine and improve guidance for meat and poultry establishments, this session will provide an update on efforts by federal agencies and their partners to fill relevant gaps and develop scientific support for lethality processes identified as scientific gaps in Appendix A. This session will also provide an overview of how industry and academic partners might develop, validate, and implement custom lethality processes as an equivalent alternative to recommendations provided in Appendix A. Overall, this session aims to provide industry with additional resources for supporting lethality processes within their hazard analyses (9 CFR 417.5(a)(1)) while highlighting areas where additional research may expand opportunities for innovation and optimization.

S40 Food Safety and Packaging Sustainability: Protecting Our People and Our Planet

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NAEEM MADY: *Intertek, Boca Raton, FL, USA*
CRYSTAL HOWE: *Ice River Sustainable Solutions, Shelburne, ON, Canada, Canada*

Political pressure and consumer perceptions regarding packaging are rapidly evolving with a strong push for corporations to have documented commitments to sustainability. Ensuring materials are sourced sustainably, support recycling, while also optimizing product quality and safety are key to meeting many sustainability goals. The packaging industry is the largest waste generator and accounts for 55% of global waste. As such, many sustainability goals are focused on tackling plastic waste. Unfortunately, this is not an easy solve for the food industry. Currently, plastic packaging is a key component in food production and in many cases essential to ensuring food safety and maintaining the integrity of the product.

In this symposium we will review the impact to food safety as sustainability goals drive the change to recyclable plastics and incorporating recyclable plastic materials within food contact packaging. The audience will hear the perspective from Canada's largest retailer as they intertwine strategic plastic reduction goals and product innovation across a vast category of control branded products. Consideration will be made to understand the various risks, controls and quantitative methods used to identify hazards associated with recycled packaging material. And finally, the audience will hear how a corporation has overcome challenges in pursuit of more eco-friendly operations in the world of plastic packaging.

S41 Bridging the Gap: From the Lab to Real-World Use

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TAURAI TASARA: *Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland, Switzerland*
UELI VON AH: *Agroscope, Bern, Bern, Switzerland, Switzerland*

Pathogens such as *Listeria monocytogenes*, *Salmonella*, Norovirus, other viruses, and fungi continue to be a problem regardless of advancements in our understanding of them. It could be that research has focused more on improving the understanding of these pathogens without a very strong focus on the application of this new knowledge to solving real-world problems. With the rising pathogen resistance to hurdle techniques and disinfection procedures as well as the frequency of food and water-borne outbreaks, it has become more important that we also focus on translating the advancement of our understanding of these pathogens to better control or avoid such outbreaks, reducing their threats to food safety and public health. Climate change, increased industrialization, intensive farming, and processing of food all contribute to altered microbe behavior and increased chances of pathogen evolution and the emergence of new more dangerous pathogens as seen with the COVID-19 virus. Moreover, pathogens are easily spread as the world has increased in its interconnectivity through travel and trade. Therefore, we need to restructure our research to also focus on applying all the brilliant laboratory findings to improve public health and food safety.

This session focuses on identifying advancements and challenges to translating laboratory findings to real-life application and aims to stimulate discussion to bring about a change in research focus and identify some of the gaps and concerns to be addressed. Key session takeaway will include the realization that (i) translational research is key to improved pathogen control and for countering microbial adaptation against most interventions. (ii) Microbes continue to evolve, likewise, research must also evolve. (iii) Controlled laboratory experiments help in developing new hurdle techniques, and generate and support hypotheses. However, implementation is often under non-controlled variable conditions which often alters the predicted efficiency of an intervention. (iv) Microbiology research must focus on both advancement of knowledge and application of gained insights to solve real-life problems.

S42 Root Cause Analysis to Identify Causes of Viral and Parasitic Diseases Outbreaks: Does It Matter?

TIM JACKSON: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
DREW MCDONALD: *Taylor Farms, Salinas, CA, USA*
JULIE JEAN: *Institut sur la nutrition et les aliments fonctionnels, Université Laval, Québec, QC, Canada, Canada*

Foodborne outbreaks caused by viral and parasitic pathogens continue to occur linked to the consumption of different types of produce. Viral Hepatitis, for instance, continues to be an important cause of human illness globally. In the U.S., several viral hepatitis outbreaks linked to consumption of fresh and frozen berry fruits have been reported in the last few years. In addition, *Cyclospora cayetanensis*, the culprit for large foodborne outbreaks of cyclosporiasis associated with ready-to-eat and other types of fresh produce, has becoming an increasing food safety concern in the U.S. and Canada. These outbreaks result from systematic failures in the food systems designed either to control known hazards or to anticipate potential novel hazards. Opportunities exist to identity the root cause of these outbreaks by applying a systematic, analytical approach known as root cause analysis (RCA). Timely initiation of an RCA

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is important because after a certain period it is practically impossible to reconstruct the factors that led to the occurrence of an outbreak. In addition, experiences accumulated from RCAs conducted in past outbreaks should contribute to refine the process and approaches to identify the true causes of a problem, as well as how to eliminate them. This symposium will discuss the different aspects of RCAs conducted to identify underlaying causes of viral and parasitic diseases outbreaks including the challenges that are encountered when trying to identify the root cause of these outbreaks.

S43 How to Use Data to Identify Key Needs and Drive Evidence-Based Organizational Food Safety Culture Change: Learnings from Dairy Industry

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DON PAGH: *Saputo Dairy Foods USA, Decatur, AL, USA*
JONATHAN FISCHER: *HP Hood LLC, Wilmington, MA, USA*
CONRAD CHOINIERE: *Office of Analytics and Outreach, Food and Drug Administration, U.S. Department of Health and Human Services, College Park, MD, USA*

Food safety culture has gained increasing discussion in the recent decade. Despite the many existing models on assessing food safety culture, there is limited empirical evidence on how exactly an organization identifies targeted action and assesses needs based on data. This data-driven culture change is especially relevant nowadays with accentuated regulation and third-party standards. Therefore, this symposium will share (1) how predictive regression and a Tableau dashboard were used on data from 86 dairy plants to identify statistically significant strengths and weaknesses and to drive changes, (2) real-world examples from industry Quality leaders based on implementing food safety culture programs at two different companies, and (3) how these food safety culture measurements and actions contribute to regulating food safety and business future from a regulatory perspective. The statistical analyses on the culture measurements and their impact on culture changes will be discussed. Improvements, challenges, and best practices from dairy industry will be shared. Regulatory implications will be examined.

From this session, the audience will learn where to start the data-driven organizational culture change, and how to leverage existing resources to prioritize key needs and create sustainable, meaningful culture change.

S44 Food Allergens in Foodservice – Detection, Control, and Management

JOSEPH BAUMERT: *University of Nebraska, Lincoln, NE, USA*
BETSY CRAIG: *MenuTrinfo, Ft. Collins, CO, USA*
GABRIELA LOPEZ VELASCO: *NEOGEN, Lansing, MI, USA*

Food allergens are a global issue with approximately 7% of the world's population having a food allergy. In the U.S., 17 out of 63 deaths related to allergic reactions from foods are restaurant related. This may be due to high turnover rate, misconceptions from team members on the severity of food allergic reactions, and lack of allergen training. Currently, only 5 states and 2 cities have mandatory allergen training for restaurant employees. Ways to reduce these incidents may by improved labeling and more risk assessments. Food allergens are present in many foods and ingredients in foodservice operations, and with many savvy customers requesting allergen-free products, restaurants need to be prepared to respond to these requests. A symposium with presentations to address the best practices to prevent allergen cross-contact, allergy training regulations, technologies to test/identify allergen residues on FCS, certifications for allergen-free foodservice locations, and examples and lessons learned from incidents with customers and allergic reactions, etc. is being proposed.

S45 Cyclosporiasis in the Americas

MARIA LUNA: *Benemerita Universidad de Puebla, Puebla, PU, Mexico, Mexico*
JORGE GOMEZ: *Universidad del Quindio, Armenia, Colombia, Colombia*
MANUELA VERASTEGUI: *Universidad Peruana Cayetano Heredia, Lima, Peru, Peru*

Cyclospora cayetanensis is a coccidian parasite that causes gastrointestinal illness in humans. Initially this parasite was primarily associated with foodborne outbreaks linked to berries. Since 2013, *Cyclospora* outbreaks in the U.S. have been associated with consumption of imported herbs and salad greens. Several countries in the Americas are considered endemic for *Cyclospora*. The microbiological safety of fresh produce during production and processing is important and its association with foodborne outbreaks needs to be better understood.

This symposium will address the similarities and differences between each geographical region including the prevalence of *Cyclospora* in humans and fresh produce and their respective challenges specific to the Americas in particular Peru, Colombia, Mexico, and USA.

S46 Assessment of the Potential Allergenicity of Foods from Novel and Alternative Sources of Protein

PHILIP JOHNSON: *University of Nebraska-Lincoln, Lincoln, NE, USA*
MICHAEL ABBOTT: *Health Canada, Ottawa, ON, Canada, Canada*
RICHARD GOODMAN: *University of Nebraska, Lincoln, NE, USA*

In recent years, an array of protein-based food ingredients from novel sources including air protein, bacterial, fungal, algal, plant, insect and cell cultured meat sources have been developed and are entering the human diet. This trend is intersecting with concerns about increased public health concerns related to food allergies on a global basis. The allergens in foods are typically proteins although some food proteins definitely have more allergenic potential than others. Some novel proteins have the potential to cross-react with known allergenic proteins. Other novel proteins have some potential to develop into novel allergens. Some global regulatory jurisdictions (European Union, Canada) have novel food regulations that, in part, mandate the assessment of the potential allergenicity of novel protein sources before they are allowed for use in foods. In the U.S., the GRAS process is used and should include an allergenicity assessment of the novel protein source. Several approaches to allergenicity assessment have been utilized, although no foolproof approach exists. This session will focus on a hazard assessment of the wide array of novel protein sources, a critique of existing approaches to the allergenicity assessment of novel protein sources, and proposals for alternative approaches.

S47 Testing for Non-Cultivable Foodborne Pathogens: Interpretation of Molecular-Based Results in the Context of Public Health Risk

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MAURICIO DURIGAN: *U.S. Food and Drug Administration – CFSAN, Office of Applied Research and Safety Assessment, Laurel, MD, USA*
JEFFERY FARBER: *Department of Food Science, University of Guelph, Guelph, ON, Canada, Canada*

The contamination of produce and agricultural water with human enteric viruses and protozoan parasites is a significant food safety concern. Outbreaks of hepatitis A virus, human norovirus, and *Cyclospora cayetanensis* continue to occur, with the riskier products being soft fruits and herbs/leafy

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greens. In response, routine surveillance and testing using non-culture-based detection methods are increasingly employed, however the interpretation of results can be tricky. The purpose of this symposium is to introduce the varied nuances of interpreting detection results for non-cultivable pathogens in food, water, and environmental samples, and the potential role of testing in information gathering and risk management. The first two presentations will focus on how the data obtained from methods commonly used for detection and confirmation of enteric virus and parasitic protozoa contamination in food and water are interpreted. These presentations will consider issues such as sample size and number; assay sensitivity and specificity; meaning of Ct values; predictive value of the test; interpretation in the context of pathogen infectivity; and confirmation considerations, among others. The third speaker will discuss how the interpretation of testing results relates (or does not relate) to public health risk, and future considerations as testing for non-cultivable foodborne pathogens becomes increasingly common. Participants will come away with a greater appreciation for the complexity of this issue, and an understanding of the global landscape that constitutes detection of foodborne pathogens which are currently not possible to culture in a laboratory setting.

S48 Estimating the Cost of Foodborne Illnesses

SANDRA HOFFMANN: *USDA Economic Research Service, Washington, DC, USA*
ALICE WHITE: *Colorado School of Public Health, Aurora, CO, USA*
BRAD GREENING: *U.S. CDC, Atlanta, GA, USA*
BRENDAN DOUGHERTY: *Public Health Agency of Canada, Guelph, ON, Canada, Canada*

Economic estimates can help decision makers and the public better understand the impact of foodborne illnesses and the resources needed to prevent them. This session will present new estimates of the cost of foodborne illnesses in the United States, estimates of how antibiotic resistance increases the cost of salmonellosis in Canada, and news designed to help state departments of public health estimate the health benefits and administrative costs of foodborne illness outbreak investigation and response.

The new U.S. cost of foodborne illness estimates are the result of a multi-year research effort by USDA Economic Research Service (ERS) and the Colorado School of Public Health. They expand the coverage of prior ERS estimates to parallel 2011 CDC estimates of the incidence of foodborne illness; provide a comprehensive update of modeling on chronic outcomes of foodborne illnesses in the U.S.; and enhance uncertainty modeling capacity. Estimates of the cost of foodborne illness have been used by researchers at the CDC to develop a tool that can be used by state departments of public health to estimate the economic savings from illnesses prevented by foodborne illness outbreak investigations and interventions. CDC also collaborated with the Colorado Integrated Food Safety Center of Excellence to develop a micro-costing tool that can be used by state departments of public health to estimate the administrative cost of conducting foodborne illness outbreak investigations. These three efforts provide essential information for public health decision making on food safety and foodborne disease response. They also provide useful information for communicating with the broader public about the impact of foodborne illness and the costs and benefits of their prevention. Antibiotic resistance is a growing problem for all infectious disease. Researchers from Public Health Canada have estimated the added cost of illness from antibiotic resistance in nontyphoidal *Salmonella.*

S49 Sanitary Design for Automation and Digital Transformation

ROBERT WALLACE: *Novolyze, Bethesda, MD, USA*
DIMITRI TAVERNARAKIS: *Mondelez International, Heraklio, Greece, Greece*
TIMOTHY RUGH: *3-A Sanitary Standards, Inc., McLean, VA, USA*

Trends for automation in food manufacturing have increased significantly recently with a 2022 survey indicating 27% of food manufacturers plan to invest in automation, robotics or AI to deal with pressures from labor or the need to become more preventative through the use of data generated through Industry 4.0 initiatives. These speakers will share details about what is driving the current automation trend, what does it mean to automate or digitize in 2023 and what sanitary design considerations are critical to protect food safety. The purpose will be to provide knowledge and tools for evaluating whether or not automation is likely to be implemented in the participants food area and what are the benefits and food safety risks to that automation. Another area will be to provide a means to evaluate the risks of new automation equipment and Industrial Internet of Things devices being used in the food industry and how to appropriately mitigate these risks. The presenters have extensive experience in implementing digital transformations, evaluating food safety risk in sanitary design and trends affecting food safety.

S50 To Eat or Not to Eat: The Utility and Challenges of Using Risk-Benefit Assessments for Decision Making in Food Safety and Nutrition

MARIA BASTAKI: *Methodology and Scientific Support Unit, European Food Safety Authority, Parma, Italy, Italy*
HANS VERHAGEN: *Technical University Denmark/Ulster University/ FSN Consultancy, Utrecht, Netherlands, Netherlands*
MATTHEW DELLINGER: *Medical College of Wisconsin, Milwaukee, WI, USA*

Foods, and diets overall, are a source of multiple risks and multiple benefits. While there are risks associated with the consumption of certain foods, failure to incorporate foods with potential health benefits into the diet may be considered a health risk. Indeed, the lack of a health-benefit can be thought of as a health-risk. Qualitative and quantitative risk-benefit assessments (RBAs) can be used to support public health decisions in food safety and nutrition by integrating health-relevant evidence from various disciplines. However, carrying out an RBA is a challenging task that requires rigorous scientific analysis, while also requiring the assessor to address uncertainty, be responsive to the needs of risk managers and provide results that are translatable to consumer advice. Strengthening the link between RBA, risk management decisions, and dietary recommendations can improve public health outcomes.

The objective of this symposium is to bring together internationally renowned scientists working on RBA to present the state of the science, including emerging methods, and their application to real life dietary advice and to highlight future needs. The context of the U.S. regulatory perspective will be briefly introduced. The first speaker will then provide the European Food Safety Authority (EFSA) perspective on RBA, including the published guidance and update. The second speaker will present emerging methods and the communication bridge between risk managers and assessors. We will end the symposium with a presentation on case studies using RBAs developed for guiding health research in fish-consuming Great Lakes Native American Communities.

S51 From Farm to Food: A New Perspective on Heavy Metals in Human Diets

ANGELIA SEYFFERTH: *University of Delaware, Newark, DE, USA*
BENJAMIN RUNKLE: *University of Arkansas, Fayetteville, AR, USA*
ASHISH POKHAREL: *Michigan State University, East Lansing, MI, USA*
FELICIA WU: *Michigan State University, East Lansing, MI, USA*
KEVIN BOYD: *The Hershey Company, Hershey, PA, USA*

Have you ever wondered where heavy metals come from or why they may be present at trace levels in some foods? This session will take a look at how heavy metals enter the food supply chain (hint: they are not added-in during manufacturing) and the impact of factors such as farming practices, growing location, soil type, and processing. To help provide context, this session will also explore dietary exposure to heavy metals based on what is known about metal uptake by specific crops from their environments and also food consumption data. Importantly, using case studies of crops with high levels of consumption, the session will explore pre- and postharvest mitigation strategies to potentially reduce the presence of metals in crops and work toward the goal of continuing to minimize dietary exposure to the lowest feasible levels. Considering the diversity of factors that affect the uptake/presence of heavy metals in foods, and the uniqueness of factors for each metal and crop, mitigation options require creative solutions that must be feasible and practical to implement beginning at the farm level. Solutions to mitigate the presence of metals will involve collaboration among scientists, growers, ingredient/ food manufacturers and policymakers. Case studies presented will address mitigations of US-produced as well and internationally-sourced crops and ingredients

S52 Building Strategies for Prevention

TIM JACKSON: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
NATALIE DYENSON: *Dole Food Company, Inc., Charlotte, NC, USA*
GRETCHEN WALL: *International Fresh Produce Association, Newark, DE, USA*

Outbreaks and recalls are the most visible indications of food safety system failures. Less visible are near misses uncovered by verification of processes, materials, growing and manufacturing environments through process measurements, inspections and sampling programs. Effective investigations and root cause analysis can provide information to develop effective corrective and preventive actions and identify open questions needing further investigation, and areas where stakeholder collaboration can be effective to drive research, develop relevant guidance, standards and training.

This symposium will highlight the role of effective investigation and root cause analysis, examples of industry and multi-stakeholder collaboration on prevention, and the current FDA initiative to work with other stakeholders to identify, develop and execute prevention strategies for new or persistent issues.

S53 Digital Transformation of Data: Trials, Tribulations, and Lessons Learned from the Healthcare Industry

ROBERT WALLACE: *Novolyze, Bethesda, MD, USA*
WENDY BIGALA: *OSI Group, Aurora, IL, USA*
GALE PRINCE: *President, SAGE Food Safety Consultants, LLC., Cincinnati, OH, USA*

There is an increased necessity for the transition from written documents and Excel Spreadsheets to digitizing data for the use of AI Technology, recalls, and government mandates for the food industry. The industry understands that the digitization of data could help us with unforeseen food safety risks and potentially reduce those enterprise risks. However, the question is how and at what cost? First, we will review, "What can we learn from the healthcare industry in advancing food safety by using AI." Second, attendees will get to hear about the "Challenges to the finish line – infrastructure, locations, and user experience" from a company currently going through the transition. Finally, we will prepare the attendees for the challenges around the safety of the data. Attendees of the symposium will walk away with a deeper understanding of the trials, tribulations, and learnings from the healthcare industry, data security, and a food manufacturing company's current transition. There is no uncertainty that the food industry will have to make the digital transformation of data to support future growth. The learnings from another industry that has transformed should help arm the food industry with the knowledge of making the successful transition.

S54 The New Codex Alimentarius Framework for Safe Water Reuse in Food Production and Processing Put to the Test in Practice for Fruit and Vegetable Food Products

KANG ZHOU: *Food and Agriculture Organization of the United Nations, Rome, Italy, Italy*
ANA ALLENDE: *CEBAS-CSIC, Murcia, Murcia, Spain, Spain*
MARCOS SANCHEZ PLATA: *Texas Tech University, Lubbock, TX, USA*

Water is an increasingly precious commodity in many geographies. Codex Alimentarius, covering 99% of the governments of the world, is for the first-time developing guidance on the safe reuse of water for food production and processing that can help address issues of water scarcity where relevant. Competent authorities around the world may adopt the new guidance in their country but will be scrutinizing the feasibility and practicality of the new guidance, especially in resource limited settings.

In the past several years, the Food and Agricultural Organization (FAO) and World Health Organization (WHO) have established a new framework for science- and risk-based decision-making on fit-for-purpose water use and reuse in primary production and food operations. This framework has been developed through the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) and the science/principles foundation of the framework and its application to the fresh produce, fishery and dairy sectors has been featured at past IAFP meetings. At these meetings, there has been broad agreement of principles but quite some discussion on practical matters, and most often concerning the management of safe water use and reuse in situations where human, expertise and financial resources are scarce.

In order to test the feasibility and practicality of the new framework, FAO and WHO will stage expert and stakeholder workshops scrutinizing three aspects:

- the efficiency of the decision-making processes/systems of the framework
- the utility of potential indicator microorganisms/agents in the context of the decision-making processes/systems
- useful microbiological criteria for fresh fruits and vegetables

Key experts involved in the road-testing events will inform the IAFP food safety community of their work and results in this symposium supported by the Water Safety and Quality PDG and the International Food Protection Issues PDG as main sponsors. However, also the Microbiological Modelling and Risk Analysis PDG, the Fruits and Vegetables Safety and Quality PDG, the Dairy Products Quality and Safety PDG and the Seafood Products Safety and Quality PDG have formally confirmed their support to the proposal.

S55 Queso Fresco-Type Cheeses – Listeriosis Outbreak Prevention Strategies

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TIMOTHY STUBBS: *Innovation Center for U.S. Dairy, Rosemont, IL, USA*
ALEX O'BRIEN: *Center for Dairy Research, Madison, WI, USA*

Queso fresco-type (QFT) soft cheeses are high-moisture, low salt, near neutral pH, fresh cheeses that require refrigeration for safety. Depending on their country of origin, some of the more popular soft cheeses include Oaxaca, Panela, Queso Blanco Fresco (Queso Para Frier), Queso Fresco, and Requeson. Consumers may find these cheeses at retailers, or in prepared dishes at restaurants. The fresh nature of these cheeses provides an ideal growing condition for pathogens such as *Listeria monocytogenes*. Recent outbreaks of QFT cheeses prepared with pasteurized milk have mainly been linked to *L. monocytogenes*. Recently, cooperative efforts among various industry stakeholders have been aimed at reducing the occurrence of outbreaks linked to QFT cheese. This symposium discusses efforts that government, industry and universities have put in effect.

Symposia

S56 Ensuring Honey Authenticity – Recent Developments

NORBERTO GARCÍA: *Apimondia, President of the Scientific Commission Beekeeping Economy and Chairman of the Working Group Adulteration of Bee Products, Buenos Aires, Argentina, Argentina*
MARISA AMADEI: *Nexco, Bueno Aires, Argentina, Argentina*
JODIE GOLDSWORTHY: *Beechworth Honey, Beechworth, VIC, Australia, Australia*

This session will be a deep dive into the many complex aspects of ensuring honey authenticity in global food supply chains. We will review what is known about honey fraud and the damage it causes to legitimate producers, agricultural production, and the environment. We will introduce the innovative work of the USP Honey expert panel to develop a *Food Chemicals Codex* honey standard and to publish the Honey Fraud Mitigation Guidance for industry. The honey standard includes explicit descriptions of honey composition and quality factors, identification methods, and additional methods that can be used to confirm authenticity. A USP expert volunteer will review this work and discuss how the standard can be implemented within food supply chains. The Honey Fraud Mitigation Guidance, which is targeted for publication in 2023, provides in-depth discussion of the various contributing factors to honey fraud and a strategy for reducing the risk of fraud. The remaining speakers in the session will provide insights into some of these contributing factors, such as the challenges of identifying a "silver bullet" for analytical testing and how market economics can both incentivize fraud and provide clues that it is happening. One of the speakers will present a case study of efforts to protect the honey market and beekeepers in Australia. This session will be a comprehensive review of the current state of honey authenticity measures, and it will be valuable to anyone who sources honey as a food ingredient or eats it at home.

S57 Optimizing Sanitation in the Produce Industry

RUTH PETRAN: *Ruth Petran Consulting, LLC, Eagan, MN, USA*
VALENTINA TRINETTA: *Kansas State University, Manhattan, KS, USA*
JUSTIN KERR: *Factor IV Solution, Atascadero, CA, USA*

The importance of an effective sanitation program is well established, but far too often we miss opportunities to reevaluate and improve our approach to cleaning and sanitizing. Many in the fresh produce industry are reconsidering their approach to sanitation considering the unique challenges that come with harvesting and packing in the field, or relatively open environments, compounded with regulatory and buyer demands. A lack of sanitation in food environments has led to foodborne outbreaks and recalls due to the increased likelihood of product contamination (e.g., cross-contamination, biofilms). The Food Safety Modernization Act Produce Safety Rule requires documentation of sanitation activities including cleaning and maintaining all non-food contact surfaces, and inspecting, cleaning, and when necessary, sanitizing food contact surfaces used during produce harvesting, packing, and holding. Clean is assessed as visibly clean in produce environments, but this raises questions: what is clean, why do we need to clean, how do we clean, what about biofilms, and how do you verify cleaning (beyond the visual inspection)? Can lessons be learned from other industries who also deal with cleaning difficult equipment, tools or environments? Picking a sanitizer is almost as personal as picking a mate, with there can be butterfly effects for packing environments; for example, adding water (wet-based sanitizers) into an otherwise dry-pack operation. Blending cleaning and sanitizing into real-life sanitation programs is easier said than done. Thus, we will dive into a real-life case study on the complexities involved in developing and implementing a robust harvester sanitation program (by the industry, driven by a food safety program need).

S58 Potentially Carcinogenic Compounds in Food and Water (Ethyl Carbamate, Acrylamide, and Chlorine Byproducts)

BENJAMIN REDAN: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
NIGEL HALFORD: *Rothamsted, Harpenden, United Kingdom, United Kingdom*
XUETONG FAN: *USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA, USA*

In recent years, numerous potentially carcinogenic contaminant compounds in foods have been spotlighted as problematic and further research is needed to determine their prevalence as well as means of remediation. Three of these compounds are ethyl carbamate (EC), acrylamide and chlorine byproducts (CBPs). EC is a contaminant by-product formed during the fermentation or processing of foods and beverages, often associated with beer, wine, and distilled spirits, and at lower concentrations in other foods products such as soy sauce, fermented dairy products, vinegar, pickled vegetables and breads. Allowable limits in alcoholic beverages have been set in several countries and animal studies have demonstrated that EC is a probable human carcinogen. Acrylamide is produced during the frying, roasting or baking of potato and grain-based foods based on the presence of sugars and asparagine. The U.S. Environmental Protection Agency classifies acrylamide as *"likely to be carcinogenic to humans."* Strides have been made to develop potato and grain crops that are more recalcitrant to the formation of acrylamide during cooking. As a result of chlorine sanitization, CBPs (such as trihalomethanes and haloacetic acids) can be generated or are a contaminant in water, fresh produce items and nuts. In addition, perchlorate is present in many fruits and vegetables. U.S. and EU regulatory agencies have legislated legal limits on maximum levels for some of these chemicals in foods and water. Further research is called for to target mitigation strategies as well as to develop rapid and sensitive methods for the detection of these compounds in the food supply. These lectures will detail these compounds, their occurrence in foods, means of ameliorating problems and analytical methods for their detection in foods and water.

Symposia

S59 Food Safety Risk Dashboards, Network Analyses, and Surveys: New Risk-Based Tools to Support Food Safety Decisions in a Global Economy

JEFFREY CHOU: *U.S. Food and Drug Administration, College Park, MD, USA*
ALBERTO GARRE: *Technical University of Cartagena, Cartagena, Spain, Spain*
JUN CHENG: *Singapore Food Agency, Singapore, Singapore, Singapore*

The confidence of consumers in the ongoing safety of their food supply depends on the ability of national authorities to identify emerging hazards and risks, implement effective controls at the national and international level, and establish effective risk communication strategies. Increasing globalization of the food supply requires governments and other stakeholders to develop and adopt advanced approaches to predict, measure and respond to potential food safety risks.

This symposium showcases several new evidence-based tools that can be used by national food safety authorities, institutes or other stakeholders to support risk-based food safety decisions. First, the United States Food and Drug Administration (FDA) will highlight their use of machine learning as a critically important tool to predict non-compliance in the global food supply to improve risk-informed resource allocation and better protect public health. Researchers from Spain associated with the Technical University of Cartagena (UPCT) will subsequently discuss advances in the use of network analyses of international food imports, exports and production as a tool to identify possible hazard sources and predict the possible spread of outbreaks. Finally, the Singapore Food Agency (SFA) will share Singapore's experience in conducting its first Total Diet Study (TDS) as the fundamental scientific evidence for their national authority to develop measures to ensure food safety and for other stakeholders (i.e., industry, consumers, etc.) to make informed food safety decisions.

The session is designed for food safety professionals from academia, industry, trade associations, consumer organizations and government interested in learning about new risk-based tools used by national authorities to guide food safety decisions.

S60 Producing Safer Sprouts: Advancements in Sprout and Seed Safety Since the Implementation of FSMA

TONG-JEN FU: *U.S. Food and Drug Administration, Division of Food Processing Science and Technology, Bedford Park, IL, USA*
CARMEN WAKELING: *Eatmore Sprouts & Greens Ltd., Courtenay, BC, Canada, Canada*
LISA MUMM: *Mumm's Sprouting Seeds, Parkside, SK, Canada, Canada*

The food industry, academia, and the government have made considerable strides in improving the safety of sprouts since the implementation of FSMA. Contaminated sprouts have been previously linked to multiple foodborne illness outbreaks. Seeds are often the source of contamination, but sprouts pose a particular concern as conditions that promote seed germination also promote pathogen growth – developing ways to minimize contamination of the seed and then the proliferation of pathogens, if present, during sprouting is crucial in the overall approach to reduce public health risks of sprouts. Additionally, in recent years, the U.S. Food and Drug Administration (FDA) has implemented the final Produce Safety Rule, conducted numerous inspections of commercial sprout operations, and published regulations and guidance documents related to the safety of seed and sprouts. This symposium will give an update on FDA's regulatory activities as they pertain to the sprout producers. The results of a new study focused on the impact of temperature on pathogen proliferation during sprouting and postharvest storage will be discussed. A sprout operation owner will give their perspective on how their industry has made seed for sprouting and sprouts safer.

S61 Preparation and Continuous Professional Development – The Essentials of Effective Food Safety Audits and Inspections

BOBBY KRISHNA: *Dubai Municipality, Dubai, United Arab Emirates, United Arab Emirates*
ANDREW CLARKE: *Loblaw Companies Limited, Etobicoke, ON, Canada, Canada*
ERICA SHEWARD: *Global Food Safety Initiative, The Consumer Goods Forum, Levallois-Perret, France, France*

Food Safety audits and inspections provide a much-needed verification step of food business compliance programs, globally hundreds of thousands of these activities are completed with the intent of providing assurance that implemented food safety programs are working effectively and food for consumers remains safe to eat.

Despite often multiple layers of oversight, including 3rd Party food safety certification audits and regulatory based inspections, significant food safety issues continue to be reported. Several high-profile recalls have been linked to pathogen contamination and reported illness, Blue Bell Ice Cream for example and more recently Abbott Nutrition which experienced a product recall linked to infant formula despite maintaining a GFSI certificate. The question remains, why are these program gaps present and going undetected and why are stakeholders and the public lacking confidence in 3rd party food safety audits.

The symposium aims to answer these questions by transparent discussion of the challenges faced in the audit/inspection world. While auditor/inspector competence continues to be an issue, the challenges faced during the recent COVID pandemic found unprecedented hurdles and issues with remote food safety audits and inspections. We will hear from a regulator on some ground-breaking data management solutions which support continued oversight, reducing the need for inspection while also aiding inspections when they are carried out. Additionally, an overview will be provided on one of the GFSI Race to the Top Features, providing an overview of the proposed framework which has been developed for auditor training and continuing professional development. Finally providing a transparent career path for individuals wishing to become a food safety auditor while recognising the work professionally. Finally, there will be an overview of the development and implementation of a retailers second party global vendor audit program, the challenges faced with using multiple auditors and information as to how consistency has been achieved.

S62 U.S. Army Funded Research in Food Safety

SHANNON MCGRAW-MANZA: *U.S. Army DEVCOM Soldier Center, Natick, MA, USA*
BOCE ZHANG: *University of Florida, Gainesville, FL, USA*
JASON BOES: *Colorado State, Fort Collins, CO, USA*

The U.S. Army Combat Feeding Division's mission is to provide operationally relevant research and development to deliver solutions for evolving field feeding challenges for the American Warfighter. Within that division, the Food Protection and Innovative Packaging Team (FPIPT) is responsible for protecting warfighter health by the prevention, detection, and elimination of food contaminants through the development of procedures, validation of novel diagnostics, analysis of risk assessment tools and validation of novel pathogen reduction methodologies. They also conduct research to provide the warfighter with rations that will ensure high levels of performance and quality, nutrient retention, and safety.

Current efforts include collaborations with academia, industry, and other government organizations to develop and transfer technology that will enhance food safety and protect the health of the deployed Warfighter. These food safety efforts include: rapid identification technology for food and water pathogen viability, testing of foodservice active sanitation technologies, and the development of cost effective sensors for the rapid screening of complex food & water matrices as a presumptive test to justify confirmatory testing.

The purpose of this symposium is to introduce the IAFP community to these research efforts as well as the U.S. Army's short and long term goals for food safety in field feeding. This session can serve as an opportunity to expand outreach and foster increased collaboration in trying to meet the unique food safety capability requirements needed for the modern Warfighter and the rest of the food safety community.

S63 Deploying Genomic and Metagenomic Tools to Tackle Animal Food Safety Challenges

STEVEN RICKE: *University of Wisconsin, Madison, WI, USA*
RYAN MCDONALD: *FDA/CVM, Laurel, MD, USA*
JOE HEINZELMANN: *Neogen Corporation, Lansing, MI, USA*

Genomic and metagenomic technologies are rapidly expanding to become part of the common toolbox indispensable for food safety researchers, regulators, and industry personnel to better ensure product safety. The use of these technologies in the animal food (pet food, animal feed, raw materials and ingredients) area is a more recent development compared to that in the human food area. Nonetheless, researchers have been exploring such technologies to better characterize the microbial safety risks and antimicrobial-resistant organisms in animal food commodities. Industry has been capitalizing on these technologies to gain a better understanding of resident organisms and transient ones in their facilities. Regulators are starting to adopt genomic tools in responding to animal food contamination events. The symposium seeks to highlight this emerging trend in animal food safety arena. Expert speakers from academia, government, and industry will address three core topics. First, what is the status on applying genomics and metagenomics tools in animal food safety research and development? Second, how do we strengthen such applications to reach better animal food safety outcomes? Third, what challenges do we face for the wider adoption of these technologies in advancing animal food safety in universities, government agencies, and corporates. Symposium attendees will leave with a greater understanding of what genomic and metagenomic technologies are being explored to address animal food safety research and development questions, how they are used in the industry real-world to safeguard animal food production, and how regulators are adopting these technologies in day-to-day work to support animal food surveillance and compliance activities.

Note: One speaker Ryan McDonald is a junior scientist and first-time IAFP attendee. Three speakers represent industry, academia, and government, respectively.

S64 Investigating Ambiguous Outbreaks and Adverse Events

CRAIG HEDBERG: *UMN School of Public Health, Minneapolis, MN, USA*
TIM JACKSON: *U.S. Food and Drug Administration-CFSAN, College Park, MD, USA*
MICHAEL VASSER: *CDC, Atlanta, GA, USA*
MITZI BAUM: *STOP Foodborne Illness, Chicago, IL, USA*

The FDA will issue a public health advisory for investigations that have resulted in specific, actionable steps for consumers to take to protect themselves. However, outbreak and adverse event investigations that do not result in specific, actionable steps for consumers may or may not conclusively identify a source or reveal any contributing factors. FDA maintains a list of outbreak and adverse event investigations managed by their CORE Response teams. Adverse event investigations rely on self-reported data. Although these reports may name a particular product, FDA will only indicate a product and will not publicly name a specific product until there is sufficient evidence to implicate that product as a cause of illnesses or adverse events.

When investigations into the outbreaks or adverse events do not identify a pathogen or cause of the self-reported illnesses, despite extensive testing for numerous potential microbial and chemical adulterants, what learnings can be taken from these types of investigations that could contribute to a prevention strategy? These include improved investigational approaches and tools, use of Root Cause analysis to uncover factors that could improve attribution, improved outbreak communication strategies for reaching consumers and industry, and new areas of research.

S65 South-South Symposium – Learning from Large Scale Food Safety Interventions in Wet Markets of Africa and Asia

HUNG NGUYEN: *ILRI, Nairobi, Kenya, Kenya*
HIMADRI PAL: *Natural Resources Institute, Chatham, United Kingdom, United Kingdom*
GENET GEBREMEDHIN: *GAIN, Addis, Ethiopia, Ethiopia*
SILVIA ALONSO: *International Livestock Research Institute, Nairobi, Kenya, Kenya*

More than 90% of the global health burden of foodborne disease is borne by the global south. And most of this illness is the result of consumption of fresh foods sold in informal, poorly regulated markets. In many countries, public marketplaces (called wet markets in Asia) are important suppliers of fresh foods. After many decades of neglect, the last decade saw a range of food safety interventions aiming at sustainable and scalable improvements to food safety in wet markets. In particular, the International Livestock Research Institute and partners have pioneered market-based approaches based on four essential elements of promoting enabling regulatory environment; training vendors in food safety and business skills; providing simple, cheap technologies; and, most importantly, ensuring motivations are in place for behaviour change. The underlying premise is that professionalizing rather than criminalizing informal market actors improves food safety outcomes while at the same time improves nutrition and protects and enhances important sources of income and employment for the poor, especially poor women. The approach emerged from research on smallholder dairy production and marketing in Kenya and has been recently applied to market vendors in east and west Africa, India, and southeast Asia. These interventions have been rigorously evaluated using different methodologies including randomized controlled trials and double difference evaluations, and their impact on consumer and vendor knowledge, attitude and practice measured as well as reduction in risk as the result of interventions. This symposium brings diverse voices to report on 6 major market interventions in Nigeria, Burkina Faso, Kenya, Ethiopia, Cambodia, India and Vietnam.

S66 Beyond Aflatoxin: Mitigating Mycotoxin Risks in Animal Food, Feed and Pet Foods

JASON VICKERS: *Mars Petcare, Franklin, TN, USA*
STEPHANIE ADAMS: *Cargill, Wayzata, MN, USA*
IAN SCHUETZ: *R-Biopharm, Washington, MO, USA*
ANTHONY ADEUYA: *U.S. Food and Drug Administration / Center for Food Safety and Applied Nutrition, District of Columbia, DC, USA*

Mycotoxins are fungal metabolites toxic to mammals, accumulate in grain commodities and foods, and pose threat to health of people, pets, and livestock. Preventing or minimizing mycotoxins in grains and derivatives is a great challenge for animal and petfood manufacturers. Global trade, rapid climate changes, and supply chain constraints contribute to increased occurrence and complexity to mycotoxin prediction models. Grains are contaminated by multiple mycotoxins, but little is known about interactions between various toxins and, not all mycotoxins of significance are considered in most regulations or guidelines. For animal and pet food producers increased vigilance and regular testing beyond aflatoxins is necessary, considering potential adverse impacts of other prevalent mycotoxins. Global concerns about rising grain mycotoxins levels have driven innovations for more reliable testing methods, including multi-array tests for a range of mycotoxins. Companies must prevent introduction of mycotoxin-contaminated grain from entering their production facilities at levels exceeding regulatory or company-specific standards, as it cannot be eliminated once it is in the ingredients. This requires detecting mycotoxins in early stages of grain production systems by a deliberate pre harvest approach, rigorous sampling and monitoring of incoming grains and safe storage to keep mycotoxins within acceptable levels in the finished products. Therefore, it is necessary to implement a comprehensive integrated mycotoxin management program involving grain suppliers and manufacturers to minimize human and animal health risks, reduce productive loss and costs.

This symposium articulates essential pillars of an end-to-end mycotoxin management strategy: 1. Understanding sourcing risks and building strategic supply with integrated grains management program (pre-harvest field management and post-harvest storage management); 2. Novel diagnostics, analytics and other creative mitigation steps in post-harvest supply chains, 3. Risk characterization and implications of mycotoxins beyond aflatoxins. 4. Current regulatory governance, emerging policy guidance in North America and global alignment on mycotoxin risks and impacts of climate, conflicts and crisis on availability of clean grains and grain by-products.

S67 How to Engage Diverse Populations with Culturally Competent Campaigns

BRIAN HARRISON: *Health Canada, Ottawa, ON, Canada, Canada*
MARTIN DUPLESSIS: *Health Canada, Ottawa, ON, Canada, Canada*
BARBARA CHAMBERLIN: *New Mexico State University, Las Cruces, NM, USA*
CINDY JIANG: *McDonald's Corporation, Woodridge, IL, USA*
DEVLON JACKSON: *University of Maryland, College, MD, USA*

Traditionally in the U.S., food safety materials, assessments, and educational campaigns have been developed in the English language, targeting primarily English-speaking, Caucasian audiences. We know that the world is an incredible community of people that speak languages other than English and that experience food differently due to cultural traditions.

Although much of the food safety information for consumers is similar globally, there are fundamental differences in the approaches that specific countries, organizations, and educators communicate food safety to their own target populations. Beliefs, communication styles, and other cultural factors affect how individuals receive, understand, and respond to food safety information.

A "one-size-fits-all" approach is not effective in reducing the risk of foodborne illness. Not all populations resonate with certain consumer food safety practices which may prevent food handling behavior change. Developing culturally competent communications content requires educators to evaluate factors such as language, education level, and cultural differences.

In this symposium, panelists will:

- Address equity challenges within public health and risk communications.
- Explore case-studies from experiences of implementing culturally competent educational content.
- Share insights of the demographics of the front-line health and food safety educator as well as the types of resources they need to reach diverse communities.
- Discuss a framework for cultural considerations when creating media such as graphics and digital resources.
- Discuss social determinants and equity in developing programming for specific, diverse populations.
- Explore how future consumer research can gather qualitative data from diverse populations to understand perspectives and address communication needs.

With more diversity in our society, there is an obligation for culturally responsive, educationally appropriate, and accessible content. Together we can put these lessons into action These aspects can then be carried forth to create more equitable food safety and health outcomes.

S68 Reassess the Starting Point: Consideration of Pathogen Fitness Bias in Rapid Enrichment Procedures

LISA GORSKI: *USDA, ARS, WRRC, Albany, CA, USA*
HALEY OLIVER: *Purdue University, West Lafayette, IN, USA*
NIKKI SHARIAT: *University of Georgia, Athens, GA, USA*
TBD TBD: *TBD, TBD, AL, USA*

Traditional detection methods for low levels of objectionable organisms or pathogens in food or environmental samples rely on an initial pre-enrichment step in a permissive growth media followed by a selective enrichment for it to be detected by a diagnostic method. Some methods need to rely on multiple enrichment schemes to recover and detect the full range of a particular pathogen serotypes/variants. Recently developed enrichment schemes have emerged with a single workflow for selectively detecting microorganisms of interest. Microorganism growth in an environment is dependent on several factors, the most important being nutrient availability and growth conditions. In addition, it must overcome growth inhibitors or selective agents, if present and survive when other organisms in the environment are competing for the same resources. The enrichment procedures can potentially miss some strains that cannot grow/thrive in a particular enrichment media. There is very little discussion on how each organism might have different needs for cell repair, survival or growth and contain different fitness features. How an enrichment scheme itself might bias the successful growth and identification of pathogen phenotypes. This becomes particularly important when one is investigating a clinical outbreak or tracing consequential product contamination.

This Symposium will bring experts that have investigated this critical aspect of organism survival based on prevalence of common foodborne pathogens such as *Salmonella, Campylobacter* and *Listeria*. A better understanding of this initial step of microorganism growth, in commonly used diagnostic assays,

will be vital to continual improvement of the workflow for accurate pathogen isolation and identification. How genetic and external environmental factors contribute to growth fitness and survival? Is there a necessity to have multiple lines of enrichments to factor for the organism fitness bias in certain environments, especially when trying to investigate and track outbreak strains? Increased understanding of pathogens survival variations will help accurately identify the pathogen of interest and improve identification of objectionable organisms or in foodborne illness traceback investigations.

S69 Food Safety of Infant Foods: Care for Our Most Precious

KAH YEN CLAIRE YEAK: *Wageningen University, Wageningen, Gelderland, Netherlands, Netherlands*
SARA BOVER-CID: *IRTA (Institute of Agrifood Research and Technology). Food Safety and Functionality Program, Monells, Girona, Spain, Spain*
KALLIOPI RANTSIOU: *Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (TO), Italy, Italy*

Infants are more vulnerable to foodborne diseases. To ensure food safety, in general but even more so for infant foods, it is relevant to identify and rank hazards, to control the risk by properly validated interventions and to test for the hazards in the food and food processing environment for verification. In this symposium, all three aspects will be addressed by presenting the work carried out in the framework of the SAFFI project (Safe Food for infants in the EU and China). An approach using a variety of databases on hazards, foods, outbreaks, and epidemiological data is used to develop a decision support system for hazard identification and risk assessment. The impact of emerging processing and preservation technologies on the behaviour of prioritized pathogens in baby food is assessed. A decision support system prototype will be presented for setting the conditions of non-thermal processes to control hazards in fruit purees as case-study. For verification of control, studies on traditional and molecular techniques relating ingredients, environmental and end products are analysed and correlated. The work performed supplies crucial information for use in setting efficient monitoring and sampling strategies at operational (infant food companies) and governmental (food safety agencies) level, designing or evaluating HACCP programs, performing quantitative risk assessments, and for example auditing activities and are thus relevant for food industry, governments and academia.

S70 Tools Fit for the Task: Water Technical Forum to Support Risk-Based Agricultural Water Assessments

ELIZABETH BIHN: *Cornell University, Ithaca, NY, USA*
DON STOECKEL: *Cornell University, Sacramento, CA, USA*
ERIC HANSEN: *Hansen Farms, Stanley, NY, USA*
PATRICK HARTMAN: *Hartman Blueberries, Lakota, MI, USA*

The Food and Drug Administration (FDA) proposed revision to subpart E: Agricultural Water in 21 CFR 112 (Food Safety Modernization Act, Produce Safety Rule) represents a monumental departure from FDA's prior approaches to water quality evaluation. In the proposed revision, farms that use agricultural water on covered produce, prior to harvest, will shift from making decisions based primarily on water quality laboratory tests (e.g., quantitative tests for generic *E. coli* compared to standards). Instead, farms will make water-use decisions based on a qualitative assessment that may include laboratory results as one among several inputs.

Some comments to the FDA docket celebrated the distancing from water testing requirements. Commenters also voiced concern that the current proposed rule is vague and provides limited structure on how growers will implement the new system, known as an Agricultural Water Assessment (AgWA). Challenges include limited science available to support this type of qualitative assessment across growing regions, weather conditions, commodities, and prevailing land uses. Growers also may not have the microbial and risk-based expertise to accomplish the AgWA. The AgWA provides flexibility to accommodate scientific development and a diversity of risk reduction approaches, but leaves both growers and regulators without clarity about what compliance looks like.

The proposed compliance dates for subpart E represent a legal and economic cliff for produce growers in the U.S. and exporters to the U.S. This symposium aims to help resolve this problem by providing examples of risk-based approaches that could allow growers to implement the AgWA and thereby enhance public health protections. The symposium further provides growers a platform to contribute to discussion of aspects of the example approaches that are effective, identify challenges that still exist, and move toward practical end-user outcomes.

Note to Reviewers: We agree with reviewer feedback that the novelty and substantial value of this proposed session resides in the applied discussion that includes the regulated community (actual produce farmers). This modification to our pre-proposal requests a two-hour time slot if at all possible to enable two talks (one to describe the need and challenges represented by AgWA, the second to provide an overview of the tools that can be used to achieve an AgWA) and a full hour of dialog among SMEs including at least two representatives of covered farms.

The discussion points in this symposium may provide a starting point for a *Food Protection Trend*'s article.

S71 Educating and Protecting the Next Generation of Consumers: Key Needs and Opportunities for Food Safety Outreach Among Children, Youth, and Their Caregivers

KUNNA FAAL: *Michigan State University, East Lansing, MI, USA*
MARYBETH HORNBECK: *University of Georgia Cooperative Extension, Conyers, GA, USA*
SANJA ILIC: *The Ohio State University, Columbus, OH, USA*
ELLEN EVANS: *ZERO2FIVE Food Industry Centre, Cardiff Metropolitan University, Cardiff, United Kingdom, United Kingdom*

Safe food handling practices at home are often driven by cultural background, social influences, and habits formed as consumers learn to handle, prepare, and cook foods. Once habitual food-handling practices are formed, it becomes increasingly challenging to change consumers' behaviors through food safety education. Therefore, educating children, youth, and parents of infants who can continue to role model safe food handling as their children grow at home is critical to teach and promote safe food handling practices before potentially unsafe habits are formed and engrained. This symposium will explore key needs, opportunities, challenges, and success strategies on how to provide food safety education to children, youth, and parents of infants from multiple perspectives. A diverse group of speakers will speak about their experiences and research findings on implementing cooking classes and food safety education for youth in the United States, evaluating food safety programs for primary students in The Gambia, developing food safety curricula for youth with visual impairments in the United States, and safe food-handling practices of mothers that express breastmilk for infants in the United Kingdom. A multidisciplinary perspective to this issue will be provided along with discussion of key research and policy gaps and recommendations.

S72 Progressing the Field of Parasite Genomics to Improve Food Safety

LIHUA XIAO: *College of Veterinary Medicine, South China Agricultural University, Guangdong, GA, China, China*
REBECCA GUY: *Public Health Agency of Canada, Guelph, ON, Canada, Canada*
KAREN SHAPIRO: *UC Davis, Davis, CA, USA*
JENNY MALONEY: *USDA, ARS, Beltsville, MD, USA*

Advances in genomic sequencing have ushered in a new era for the epidemiology and control of pathogens with roles in food and water safety. The availability and analysis of whole genome sequencing of multiple strains and isolates of individual pathogens have yielded significant improvements in our ability to study, track, and control these organisms. However, parasites lag their bacterial counterparts in both genomic data availability and benefit realization. This lag is due to the many technical challenges that face researchers seeking to sequence, assemble, annotate, and compare parasite genomes. Parasites with roles in food and water safety such as *Cryptosporidium*, *Cyclospora*, *Toxoplasma*, and *Giardia* vary greatly in their genetic composition, but they share many of the same data acquisition challenges. Isolates of these parasites are difficult to obtain because methods for amplification range from extremely difficult to impossible. This lack of access is an important limiting factor in obtaining high quality genomes from the multiple species, genotypes, and strains needed for comparative analysis studies. Furthermore, even when the genetic material needed for quality sequences can be obtained, processing sequencing data presents with unique challenges. The streamlined analysis pipelines available in the bacterial community are often not an option for the parasite community, and custom analysis pipeline development is time consuming and often requires crosstalk between multiple biological disciplines. Thus, despite significant advances in and availability of sequencing technology and genomic analysis, the field of parasite genomics continues to exist as an emerging field. This symposium will bring together both government and academic researchers from around the world with expertise in the genomics of food safety parasites to share experiences, successes, and perspectives in the production and analysis of parasite genomic data. This symposium will also serve as a forum to discuss ideas and strategies on how genomic data can be leveraged to improve food safety through novel strategies for parasite detection, tracking, and control.

Roundtable Abstracts

SS1 Second Get-Connected Market: Connecting IAFP Professionals on Food Safety in Africa Even Better!

BARBARA KOWALCYK: *The Ohio State University, Center for Foodborne Illness Research and Prevention, Translational Data Analytics Institute, Columbus, OH, USA*
ADEWALE OLUSEGUN OBADINA: *Federal University of Agriculture, Abeokuta, Abeokuta, Ogun State, Nigeria, Nigeria*
KEBEDE AMENU: *Addis Ababa University, Bishoftu, Ethiopia, Ethiopia*
ABDOULIE JALLOW: *Food Safety & Quality Authority of the Gambia, Serre Kunda, KMC, Gambia, Gambia*
JOYCE THAIYA: *Ministry of Agriculture, Nairobi, Kenya, Kenya*

The African continent is enormously large and includes 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 an active IAFP chapter in Africa, the African Continental Association for Food Protection.

For the IAFP Annual Meeting in Pittsburgh, this chapter and various PDGs supported an innovative event, referred to as a "Special Session" of 2 hours, that aimed to connect IAFP attendees from Africa with those from the rest of the world on the challenges to Food Safety on the African continent.

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 session held in Pittsburgh allowed for a very active, two-way interaction among about 20 professionals from Africa and others from the rest of the world. The meeting room was designed such that tables and boards were made available for those "marketeers" who wished to display their ideas or wishes concerning the needs for or support to advancing food safety in Africa in order to attract the interest of the "browsers" that found their way to the event. A small number of marketeers was chosen to kick-off the session by reflecting on the wide scope of challenges in Africa during elevator-pitch presentations.

There was quite a buzz and a lot of energy among the participants for this first edition of this Special Session. While it was an excellent first edition, the organizers believe a much larger audience of "marketeers" and "browsers" can be attracted in Toronto because there is a good basis of evidence that this new format works and there is ample opportunity to advertise more and earlier to the IAFP community about it.

The proposal for this session is supported by the International Food Protection Issues PDG, the Developing Food Safety Professionals PDG and the Committee on Control of Foodborne Illnesses.

RT1 What Could "Sharing Data" Actually Look Like in an Outbreak?

LAURA GIERALTOWSKI: *CDC, Atlanta, GA, USA*
RUTH PETRAN: *Ruth Petran Consulting, LLC, Eagan, MN, USA*
DE ANN DAVIS: *Western Growers Association, Pacific Grove, CA, USA*
ANETT WINKLER: *Cargill, Inc., Unterschleißheim, Germany, Germany*
KARI IRVIN: *U.S. Food and Drug Administration, College Park, MD, USA*
BENJAMIN MILLER: *The Acheson Group, Northfield, MN, USA*

When a foodborne outbreak associated with a commercially distributed food occurs, industry and government have a shared interest in determining the cause to restore confidence in the food supply, limit the risk of ongoing illness, and protect public health. While this shared interest exists, the incentives for data and information sharing between industry and the regulatory and public health agencies are often not aligned. Industry is concerned about liability and regulatory actions, and regulatory and public health agencies must navigate an antiquated, complex patchwork system of government data-sharing regulations. Because each party in the investigation operates with partial information and without alignment of the collective expertise of industry, investigators, and epidemiologists, the root cause often remains unidentified. Although new regulations, such as the Traceability Rule, help define minimal data standards, a consistent, well-defined process for outbreak data sharing remains elusive.

In this roundtable which will be complementary to another focused on the value of data collection prior to an outbreak, experts from industry and government will discuss solutions to move towards proactively building a more consistent, well-defined process for data sharing to leverage in outbreak investigations. The session will include examination of the potential application of learnings from other industries (e.g., airlines and crash investigations), examples of outbreaks where such data sharing may initially be easier, and food company experts discussing their challenges. We will invite participation in questions such as how incentives could be created for all sides to more openly share data; whether there are data standards that government regulators and investigators can create (such as those in the Traceability Rule) so product and epidemiologic exposure data is collected in a more timely, consistent manner; and how industry and government can have more open dialogue to identify an outbreak's root cause and prevent similar outbreaks in the future.

Once data sharing is successfully applied to specific outbreak situations, learnings from these events could be applied to larger "big data" initiatives for further public health protection.

RT2 Implementation of a Risk-Based Supply Chain Control Program – An Industry Perspective

DIANA REGE: *Land O'Lakes, Dallas, TX, USA*
SARA MORTIMORE: *Walmart, Bentonville, AR, USA*
KARLEIGH BACON: *McDonalds, Chicago, IL, USA*

Per FSMA § 117.420 (21 C.F.R. 17.410(a)(1)(2)(3)(4), "a receiving facility must establish and implement a risk-based supply-chain program for those raw materials and other ingredients for which the receiving facility has identified a hazard requiring a supply-chain-applied control*."* Hence, manufacturers need to employ adequate supply chain preventive controls to significantly minimize and prevent the identified reasonably foreseeable hazards on their incoming ingredients where appropriate, based on hazard analysis. Furthermore, manufacturers need to adopt a range of activities which may include (but not limited to): use of approved suppliers, determine appropriate supplier verification activities, reviewing relevant documentation (where appropriate), to verify the effectiveness of implementation of supply chain controls. As the supply chain becomes more complex, the greater is the cumulative risk and hence, the more stringent a receiving facility should be assessing their supplier's performance through their verification activities. In addition to present day supply chain challenges, COVID presented an opportunity to leverage virtual tools for supplier assessments. Virtual tools continue to play an important role in risk assessment with rising business costs and shrinking travel budgets. For companies that have presence in multiple countries, factors such as

Roundtable

local regulations, cultural nuances, differences in maturity of the educational systems, economic pressures and other socio-political factors create hurdles in the implementation of a risk-based supply chain control program. In this symposium, experts from manufacturing, food service and retail industry will discuss the different challenges and strategies involved in implementation of risk-based supply chain controls as well present case studies on strategic approaches and best practices to effectively design, communicate and implement science-based food safety standards to ensure safe food from a public health standpoint.

RT3 How I Learned to Stop Worrying and Love Food Chemicals: Hot Topics in Chemical Food Safety

ASHLEY ROBERTS: *AR Toxicology, Toronto, ON, Canada, Canada*
NAKIA SMITH: *The Coca-Cola Company, Atlanta, GA, USA*
JOE ZAGORSKI: *Michigan State University, Lansing, MI, USA*
STEVEN HERMANSKY: *U.S. Food and Drug Administration, U.S. Department of Health and Human Services, College Park, MD, USA*
KEVIN BOYD: *The Hershey Company, Hershey, PA, USA*

Chemicals are all around us, including in the foods that we eat every day. IAFP attendees have likely encountered news articles or social media posts warning them of the potential danger that these chemicals are posing, typically encouraging dramatic changes in the food they eat to completely eliminate these chemicals from their diet. In order to maintain a safe food supply, food toxicologists conduct risk assessments on the broad range of chemicals that could be present in food. these chemicals include naturally occurring substances like heavy metals and aflatoxins, process-formed substances like acrylamide, and substances intentionally added to food for a technological use. Food toxicology has many unique challenges such as the complexity that food matrices create for analytical methods, and the need to evaluate substances that often have limited data. In this session, a broad panel of food toxicologists will provide their insights on what makes the job of a food toxicologist challenging through examples pulled from the headlines. These examples will stimulate the audience to ask the questions they have always wanted to have answered about the safety of chemicals in food, and give the audience tools to help them evaluate warnings about food chemicals they come across in the future.

RT4 Microbial Modeling for Food Safety: What are Some of the Liability Issues?

MARK MOORMAN: *FDA, Washington, DC, USA*
MARIEM ELLOUZE: *Nestlé Research Center, Lausanne, Switzerland, Switzerland*
BALA KOTTAPALLI: *Walmart, Omaha, NE, USA*
DONALD W. SCHAFFNER: *Rutgers, The State University of New Jersey, New Brunswick, NJ, USA*
SHAWN STEVENS: *Food Industry Counsel, LLC, Milwaukee, WI, USA*

Microbial modeling has been a useful tool in the prevention of potential foodborne illnesses for over 100 years, with the first models designed to improve the safety of canned and other thermally-processed products through the prediction of process lethality. More recently, pathogen growth models improved the safety of ready-to-eat (RTE) meat products through the optimization of product formulation. Many models in use today align with guidance from regulatory bodies such as USDA FSIS, FDA, and EFSA and when used appropriately with experienced technical judgment can continue to support improvements in food safety. Perhaps because they are generally employed by skilled practitioners, models have an enviable safety record, which might be why liability issues surrounding their use have not been publicly questioned. At the IAFP Modeling and Risk Analysis Professional Development Group meeting in Pittsburgh, concerns were raised that members did not understand the potential liability issues associated with use of models. Do they create new risks for practitioners or do they mitigate liability exposure? Do they transfer risks from product developer to modeler? What practices minimize liability when models are used for decision-support? Expert panelists with backgrounds in law, modeling and risk management representing industry and regulatory perspectives will address these questions and more.

RT5 Making Your Environmental Monitoring Data Count

NICOLE MARTIN: *Cornell University, Ithaca, NY, USA*
FABIANA GUGLIELMONE: *Unilever, Group Quality Excellence, Munro, Buenos Aires, Argentina, Argentina*
BISMARCK MARTINEZ: *Del Monte, Coral Gables, FL, USA*
TIMOTHY BUISKER: *Smart Data Science Solutions, Galena, IL, USA*
LONE JESPERSEN: *Cultivate, Hauterive, Switzerland, Switzerland*

For many years, food companies have invested in the collection of food safety data which are typically used for reactive go/no-go decisions, such as "the room and equipment is/is-not clean to run," "pathogens were/were not found in the production area." After decisions are made, the data are often forgotten, hidden away in binders, providing no further value to the company.

In this roundtable, experts in food safety, food safety culture, and data science will discuss how to derive more meaningful insights from your environmental monitoring data and engage your teams and leaders in an impactful way. They will share examples of how to elevate the level of sophistication of your data to help strengthen food safety, support positive behaviors, and foster a mature food safety culture. Key themes for discussion include data quality and reliability, driving actions and behaviors based on environmental monitoring data visualization, and methods or tools that may help leadership make decisions and change behaviors.

RT6 Sanitation Deserts – Improving Sanitation Availability to Small-and Medium-Sized Produce Operations

ELIS OWENS: *Diversey, Henderson, CO, USA*
KAREN ULLMANN: *WA Department of Agriculture, Seattle, WA, USA*
DEBRA SMITH: *Vikan, Swindon, United Kingdom, United Kingdom*
ELIZABETH BIHN: *Cornell University, Ithaca, NY, USA*
BILLY MITCHELL: *Florida Organic Growers, Gainesville, FL, USA*

Fresh produce continues to be a common vehicle for foodborne pathogens. At the same time, billions of pounds of fresh produce losses are recorded each year, which can partially be attributed to spoilage. Sanitation is a key program for fruit and vegetable growers, packers, and processors to control both issues. However, availability to sanitation chemicals, equipment, services, education, and training are not equitable across the fresh produce sector. Large or multinational fruit and vegetable producers can leverage their size and experts to execute a well-rounded sanitation program. On the other hand, many small to medium sized produce growers lack the size, purchasing power, and technical expertise needed to address their sanitation challenges to meet their goals. So, why do smaller operations lack routine availability to sanitation chemistry and equipment? How does industry, academia, and associations improve availability to key sanitation program features for small and medium size growers? How can these entities improve access to

education and training for these growers? This panel will discuss underlying reasons for this disparity and paths forward for better availability and integration. This diverse panel will be composed of experts from multiple sectors who each share a responsibility to solving this important issue. They will share their perspectives, pain-points, and success stories on this issue and on how to bridge the sanitation gap between produce growers. They will also discuss opportunities for closing the training and education gap often expected at this level. Roundtable attendees will leave with a better understanding of sanitation challenges smaller operations face and ways to improve sanitation and food safety for this underserved market.

RT7 Less Than 5 Log Reduction: When is It Appropriate? A Food Industry Perspective

YVONNE MASTERS: *John B. Sanfilippo & Son, Inc., Elgin, IL, USA*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*
YUQIAN LOU: *PepsiCo, Purchase, NY, USA*
APRIL BISHOP: *TreeHouse Foods, Oak Brook, IL, USA*
MATT HENDERSON: *Land O'Frost, Inc., Munster, IN, USA*
ANETT WINKLER: *Cargill, Inc., Unterschleißheim, Germany, Germany*

Establishing performance standards in the manufacture of foods serves to ensure the safety of our food supply and protect public health. However, certain commodities and food categories are not amenable to be subjected to one specific processing step that will deliver the widely recognized 5-log reduction performance standard. Could a multiple hurdle approach that include the microbial profile of the ingredient, process and sanitation control be considered to assure food safety? Even if a food commodity will have received a 5-log reduction kill step, post process-contamination can occur if the production environment is not maintained under sanitary conditions. The 5-log or higher performance standard, therefore, should be based on the acceptable level of protection (ALOP) or Food Safety Objective (FSO) and the expected level of contamination of the pathogen(s) in raw materials or in-process materials used to manufacture a given type of food. For where process conditions that can support the growth of pathogens or toxin formation in a food commodity exist, the appropriate process controls must be applied. The availability or lack thereof of prevalence data on pathogen(s) of concern in various commodities can add complexity conducting risk assessments of the potential health risk. While some segments of the food industry such as meat and poultry, certain produce, spices and nuts, collect prevalence data of pathogen(s) of concerns, but many do not. This session will present scenarios where the less than 5-log performance standard could be applied to ensure public health based on available data.

RT8 Crunching Beneath the Shell: Demystifying Insect Protein and Risks for Food and Feed

STEFANO LUCCIOLI: *Food and Drug Administration, College Park, MD, USA*
VINAYAK GHATE: *National University of Singapore, Singapore, Singapore, Singapore*
JESSIE USAGA: *National Center for Food Science and Technology (CITA), University of Costa Rica, San Jose, Costa Rica, Costa Rica*
KELLY HAGEN: *Entomo Farms, Norwood, ON, Canada, Canada*
KEVIN BACHHUBER: *Madison Cricket Farm, DeForest, WI, USA*
PAT CROWLEY: *Chapul Cricket Protein/Chapul Farms, Salt Lake City, UT, USA*

Due to the rapidly increasing population, decreasing available land, and fluctuating agricultural and supply chain challenges due to climate change, alternative food sources continue to gain importance and interest around the globe. Insects are an emerging alternative human and animal food source due to their nutritional, environmental, and economic value, with 92% of insects currently wild-harvested. The global overall insect protein market is expected to grow by 27% yearly from $250M in 2020 to 2028.

Increased interest in entomophagy (insects as food) poses multiple food safety challenges and opportunities for food manufacturers, educators, and consumers. Challenges include unfamiliarity with the use and safety for both human food and animal feed; the mechanisms for foraging or raising, harvesting, and processing insects (terrestrial or aquatic); food safety risks (biological and chemical) and preventive controls associated with insects in food(s) and feed(s); confusion about where to find reliable resources for production and food safety best practices; and cultural biases.

In this roundtable, a panel of experts will discuss three topics surrounding insects used for human or animal food from a global perspective: basic terminology, usage, and landscape of the international market; the risks (e.g., bacterial, parasitic, toxicity of metals and pesticides, physical hazards, end-of-use, allergenicity, etc.) associated with the production and consumption of such products from feed-to-fork; and how the risks can be addressed. This discussion aims to demystify the basics of entomophagy for human and animal foods for those in the food industry, provide various international perspectives on insect consumption, and open a dialogue between experts and attendees.

RT9 Data Sharing in the Digital Age of Food Safety

DE ANN DAVIS: *Western Growers Association, Pacific Grove, CA, USA*
JAMES DOYLE: *Creme Global, Dublin, Dublin, Ireland, Ireland*
SOFIA SANTILLANA FARAKOS: *U.S. Food and Drug Administration, College Park, MD, USA*
ANGIE SIEMENS: *Cargill, Inc., Wichita, KS, USA*
MARIE BRETON: *Health Canada, Ottawa, ON, Canada, Canada*

In the modern age of food safety, more emphasis is being placed on the use of digital tools such as predictive modeling and risk assessments to improve food safety. These digital tools require large data sets to result in accurate and meaningful information that can be used to improve food safety. While this can be an expensive and time-consuming endeavor when completed on an individual company basis, this problem can be circumvented by sharing real-world data between entities. However, there is concern that without proper privacy measures taken, data can be misused leading to potential negative public and regulatory ramifications or disclosure of proprietary information. As a result, there is hesitancy to share data without more established safeguards mapped out among both regulatory and industry parties. With a proper framework in place, a shared database would be widely beneficial to enhance modeling tools capabilities creating an opportunity for Smarter Food Safety systems as described by the FDA which ultimately will improve food safety and public health. Therefore, this session first aims to establish a dialog regarding the types of data that needs to be shared for accurate modeling and how this data can be effectively used from the perspective of those that work with these digital tools. Additionally, this session will address potential mechanisms to share data and ensure privacy from both a legal perspective and from those with experiences sharing data resulting in improved food safety.

RT10 Produce Safety's Solutions: Turning Policy and Science into Action

FRANK YIANNAS: *Smarter FY Solutions, Bentonville, AR, USA*
JIM BRENNAN: *SmartWash Solutions, LLC, Salinas, CA, USA*
MICHELLE DANYLUK: *University of Florida CREC, Lake Alfred, FL, USA*
JENNIFER MCENTIRE: *Food Safety Strategy, Washington, DC, USA*

This roundtable will feature a conversation around produce safety solutions, specifically the role of turning policy and science into action. We will present several investigations from past outbreaks, while engaging the panel in a reassessment of the situation, and what are the next steps to making meaningful change, versus repeating history, or closing investigations at points of convenience. This roundtable will be unique, and promises not to disappoint, providing an overview of produce safety solutions using data from a modality of sources to describe past outbreak investigations. We will push the conversation and discussion further (beyond presentations, to engagement with the audience). Our roundtable will consist of an expert panel to briefly provide reflections and reactions with real life perspectives and experiences. Outbreak investigations, drawn from multiple commodities and granular engagements to follow root cause analysis, and more focused critical path problem solving will provide a rare open forum view into insights to hazards and risks, actions taken from these findings, system advancements from industry-led assessments, and partnership-building in research and data analytics opportunities. Audience attendees will have a unique opportunity to learn about current industry practices and the breadth of recent risk management insights achieved from pursuing the "details of details" associated with or implicated in hazard and risk hypothesis generation.

RT11 An Ever-Changing Landscape: Can Using Indicator Organisms and Run Time Validation Studies Allow Industry to Demonstrate Process Control While Maintaining Product Safety in Low-Moisture Foods?

JEFFREY KORNACKI: *Kornacki Microbiology Solutions, Inc., Madison, WI, USA*
GERARDO MORANTES: *Bühler Group, Minneapolis, USA*
NATHAN ANDERSON: *U.S. Food and Drug Administration, Bedford Park, IL, USA*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*
JOHN HOLAH: *Kersia Group, Bury, United Kingdom, United Kingdom*
BRIAN FARINA: *Deibel Laboratories, Inc., Gainesville, FL, USA*

Extended process runs are common in the manufacture of low-moisture foods that operate without frequent sanitation breaks. Clean breaks should be incorporated into the process at some point; however, what is a systematic approach to using them as preventive interventions? What are they, how can frequency be determined, and ultimately what is gained by using them proactively?

Common cause failures like soil build-up and the creation of biofilms during extended runs can harbor pathogens potentially compromising public health. Can industry be more proactive by using microbiological data such as indicator organisms that suggest conditions have changed in the equipment or environment that might warrant cleaning interventions and provide evidence of process control?

What would drive the use of clean breaks that allow for batch-to-batch microbiological control is now a topic of debate. Do economics, the cost of downtime for cleaning versus recall costs, or performance should a loss of efficiency be observed due to reduced heat transfer or pressure drops factor into the decision?

How can the concept of clean breaks and their judicious use be employed by the low-moisture foods industry to substantiate process control?

Topic for discussion include:

- Clean breaks – a global desire – what are they?
- When is a clean break required – what are the drivers?
- How can we use microbiological indicator organisms to signal the necessity for a clean break? Can indicator organisms provide evidence of process control?
- Do we have evidence that extended run-times are a common cause of product safety failure?
- What are the key microbiological indicators of creation of growth points in the process?
- How do you set up a run time validation study? Study design? Parameters measured? Sampling locations?
- Does an increase in key indicator organisms equate to a higher risk for OOS results for pathogens?
- What process changes could be implemented to allow for the incorporation of clean breaks?

RT12 The Importance of Diversity in Building Large Integrated Food Safety Initiatives and Projects

MARK CARTER: *U.S. Department of Agriculture – NIFA, Washington, DC, USA*
JOSEPH ODUMERU: *Ministry of the Environment, Etobicoke, ON, Canada, Canada*
BYRON CHAVES: *University of Nebraska-Lincoln, Lincoln, NE, USA*
CATALINA LOPEZ CORRERA: *Genome Canada, Ottawa, ON, Canada, Canada*
MICHELLE DANYLUK: *University of Florida CREC, Lake Alfred, FL, USA*

The advancement of food safety science over the past two decades has moved researchers in the direction of multidisciplinary teams comprised of individuals from a wide variety of institutions, agencies and industries, who collectively analyze large datasets that need to be pieced together to create a picture. Having perspectives from a variety of backgrounds, experiences, cultures and home disciplines allows for a more complete and synergistic look at the food safety and research challenges that face our food protection world. Purposeful diversity in gender, culture, race/ethnicity, sexual orientation and career stage can grow stronger more rounded teams. These progressive projects and approaches do not come without barriers, however. Roadblocks, especially cultural, experiential and practical ones, often can be fatal flaws in this team approach and it is incumbent on the food protection community to wade through the barriers and foster diverse teams, regardless of the difficulties. Emerging scientists will continue to exist in a world of creating and coordinating teams with diverse perspectives and this round table will provide a medium for those on diverse, large project teams to discuss their lessons learned through their experiences.

RT13 Practical Approaches to Compliance with the Intentional Adulteration Rule, Benchmarks and Challenges

JAMES DOYLE: *Creme Global, Dublin, Dublin, Ireland, Ireland*
COLIN BARTHEL: *U.S. Food and Drug Administration, College Park, MD, USA*
LORALYN LEDENBACH: *Kraft Heinz, Chicago, IL, USA*
FRED SODERSTROM: *Unilever, Chicago, IL, USA*
SHAHRAM AJAMIAN: *McCormick and Company, Marietta, GA, USA*
YVONNE MASTERS: *John B. Sanfilippo & Son, Inc., Elgin, IL, USA*

2023 will mark four years since the announced FDA FSMA Intentional Adulteration (IA) Rule compliance date for large businesses. The IA Rule focuses on the highest risk with intentional adulteration of food to prevent wide-scale public health harm. Many businesses seek additional information to properly perform assessments and implement strategies to comply with the regulation. This roundtable is intended to provide a roadmap toward compliance with the IA rule by sharing best practices and examining challenges.

Specific topics related to compliance with the IA rule include a comprehensive training strategy that is inclusive of supervisors and frontline workers to understand the breadth of the IA rule and its importance; how to develop a vulnerability assessment that identifies significant vulnerabilities and actionable process steps to prevent intentional adulteration; how to design and implement practical mitigation strategies to limit or prevent intentional adulteration; requirements for food defense plan documentation; best practice on data management; challenges with the IA rule compliance; and FDA's observations and expectations with the rule implementation.

The panel includes senior industry leaders with responsibility for managing IA within their organization, representatives from the regulatory community, and experts from the data science community. The new requirements create opportunities to share executional examples from the industry to help companies improve the evaluation of threats and implementation of mitigation strategies. It is equally critical to share feedback from FDA on observations the agency has so far to support effective roll out of the rule. This roundtable will also serve as a platform to share challenges and provide feedback between industry and the FDA on the areas that the rule may need further clarification or guidance. Those who attend this session will gain best practices for compliance, strategies for improving food defense plans, and understanding current challenges across the industry.

RT14 Produce Safety Education and Extension Outreach Efforts Targeting Spanish-Speaking Communities in the United States

AFREEN MALIK: *Western Growers Association, Irvine, CA, USA*
SERGIO NIETO-MONTENEGRO: *Food Safety Consulting & Training Solutions, LLC, El Paso, TX, USA*
VALENTIN SIERRA: *Amigo Farms, Inc., Yuma, AZ, USA*
JACQUELINE GORDON: *Washington State Tree Fruit Association, Yakima, WA, USA*
ALEXANDRA CORTES: *Minnesota Department of Agriculture, Minneapolis, MN, USA*

Spanish-speaking communities in the United States play an important role in the fresh produce industry today. They make up a significant portion of the farm workers, owners, and managers in the localities where fruits and vegetables are produced, packed, and held. However, because of the linguistic and socio-economic disadvantages faced by many Spanish-speaking communities across this country, this important segment of the fresh produce industry lacks the same level of accessibility to high-quality food safety information, education, and training resources than other groups. Thus, establishing effective ways to reach out to Spanish-speaking farmers through community-specific extension training programming is essential to help them understand and manage food safety hazards in their farms or workplaces. Although the use of Spanish language is an essential first step to increase access to food safety training and resources, merely translation of existing training materials and resources from English to Spanish will not effectively reach and train these communities. Translated training materials and resources should be accompanied by other outreach strategies specifically tailored to the different Spanish-speaking communities found throughout this country.

Therefore, this roundtable will provide an opportunity to share experiences regarding different approaches used to reach and train Spanish-speaking communities across the produce industry in the United States. Our discussion will focus on sharing different perspectives, empirical and research-based results, and practical recommendations regarding the appropriate communication and outreach channels to effectively reach and train the Spanish-speaking communities. Additionally, we will exchange suggestions on how to build effective working relationships based on cultural awareness and understanding with colleagues from extension, academia, and the industry. Because of the great diversity in geographic, cultural, and socio-economic realities found in the Spanish-speaking communities in the produce industry, it is important for educators to continually improve our understanding of the needs of these communities to better serve them.

RT15 Are Rapid Methods Dead? What Methods Does Industry Really Need in the Current Climate?

PURNENDU VASAVADA: *University of Wisconsin-River Falls, River Falls, WI, USA*
CATHARINE CARLIN: *Mérieux NutriSciences, Chicago, IL, USA*
JOSEPH MEYER: *Kerry, Waunakee, WI, USA*
PAMELA WILGER: *Post Consumer Brands, Lakeville, MN, USA*
DOUGLAS MARSHALL: *Eurofins, Fort Collins, CO, USA*
DANIELE SOHIER: *Thermo Fisher Scientific, Dardilly, France, France*

Microbiological testing of raw material, ingredients, food plant environment and food products is critical and essential part of the quality and safety strategy of food industry. Conventional testing methods, while considered "Gold Standards" in many cases, are time, material and staff intensive, retrospective and not always appropriate for challenges of emerging and hard to detect and grow organisms. Thus, there has always been tremendous interest in Rapid methods and automation in food microbiology.

Over the past several decades, there have been many developments in microbiological testing methods for enumeration and detection of organisms of concern. But over that time there have been dramatic changes in how testing is done and the need for different test methods is changing. Many industries that once relied on their own laboratories to do testing, now send samples to external laboratories. The technical skill base in many laboratories has also changed, with fewer "expert microbiologists" and more "technical operatives" highly capable of doing laboratory procedures but perhaps less skilled at reading "subjective" end points results. We have changes in what analyses companies may wish to do, unculturable pathogens such as viruses, and pathogenicity genes such as stx may be future analytical needs. Additionally, the whole focus of risk management has moved from testing safety into foods, to designing safety into foods via risk assessment and implementation of HACCP.

In this new arena, do companies really need a primary goal of perhaps costly "rapid methods". Or do they need economically priced, highly automated methods with very objective end points, that may not have speed as their sole objective.

With input from method producers, testing laboratories and food producers, this session will explore the area in depth, looking forward to future industry testing needs.

RT16 Consumer Food Complaint Systems: New Approaches, New Insights – and Potentially New Risks – with a Conventional Food Safety Surveillance Tool

ELAINE SCALLAN WALTER: *University of Colorado, Denver, CO, USA*
NATHANIEL WILSON: *Kentucky Department for Public Health, Frankfort, KY, USA*
MARIJKE DECUIR: *Minnesota Department of Health, St. Paul, MN, USA*
LORRAINE HASKINS: *Canadian Food Inspection Agency, Ottawa, ON, Canada, Canada*
NOËL HATLEY: *Washington State Department of Health, Olympia, WA, USA*
OLUWAKEMI ONI: *Iowa Department of Public Health, Des Moines, IA, USA*

Consumer complaint systems are a cornerstone of foodborne disease and adverse event surveillance. Complaints have historically been by far the most common mechanisms for outbreak detection. Consumer complaints are also the primary mechanism for detecting outbreaks caused by etiologic agents that laboratories seldom analyze or where laboratory methods are lacking. Whether a detected outbreak is investigated, and a cause of the outbreak is ultimately found, however, requires rapid collection and assessment of actionable information and prioritization. Increasingly, states are implementing more centralized systems and on-line reporting that provide a single point of entry for consumers and aggregate complaints across several local jurisdictions, providing a bigger, clearer picture and helping identify outbreaks that may have been missed. State systems have also incorporated automated workflows that prioritize complaints based on keywords and characteristics and send alerts for follow-up. Increasing aggregation and ease of reporting by consumers has its caveats, though. Crowd-sourced complaint systems, social media trending, and widespread media publicization increase the risk of pseudo-outbreaks where hundreds of consumer complaints mention the same food product or brand but lack a true causal relationship. This roundtable session will explore the improvements in complaint system technology, discuss novel outbreaks triggered by complaints, and identify what complaint systems excel at and where they are vulnerable.

Notes for Program Committee: Updates have been made to panel member list. All panelists have been notified and are willing to participate. Potential overlap with 8488 has been discussed with the organizer and mitigated.

Roundtable

RT17 Animal Feeding Operations, Environmental Hazards: Problems, Solutions, and Incentives

DAVID GOLDMAN: *Groundswell Strategy (retired USDA), Washington, DC, USA*
DE ANN DAVIS: *Western Growers Association, Pacific Grove, CA, USA*
SHIRLEY MICALLEF: *University of Maryland, College Park, MD, USA*
NIKKI SHARIAT: *University of Georgia, Department of Population Health, Athens, GA, USA*
MICHELE JAY-RUSSELL: *Western Center for Food Safety, University of California, Davis, CA, USA*

A review in the August *Food Protection Trends* highlighted food safety hazards posed by proximity of produce growing operations to animal feeding operations (AFO). There are additional papers in the past five years also describing the hazards related to mixed agricultural food systems. AFOs can be a source of zoonotic contamination to the environment, and thus fresh produce, and meat and poultry products. For instance, in February FSIS wrote, "Currently, events that cause contamination of pork carcasses cannot be completely eliminated from commercial slaughter, fabrication, or further processing operations." FSIS data show that the problems in poultry are worse. The zoonotic hazards enter FSIS inspected facilities on animals and exit on raw product.
There are solutions. The National Academy of Science in 1970 JMFT Summary of the 1969 report*, included a list of interventions. One committee member, Pomeroy, implemented them to produce *Salmonella*-free turkeys. Since then, scientists have developed many more interventions including prebiotics, probiotics, vaccines, and bacteriophages.

What is lacking is incentives. This roundtable will discuss the problem, solutions, and incentives.

*Committee on *Salmonella*, National Academy of Sciences–National Research Council, 1970. An Evaluation of the *Salmonella* Problem: Summary and Recommendations. *J. Milk Food Technol*. 33:42-51.

RT18 Lost in Translation: Advancements and Challenges to Translating Laboratory Findings to Real-Life Application

KATHLEEN O'DONNELL: *Wegmans Food Markets, Inc., Rochester, NY, USA*
TOM ROSS: *University of Tasmania, Hobart, Tasmania, Australia, Australia*
ANA ALLENDE: *CEBAS-CSIC, Murcia, Murcia, Spain, Spain*
LYNN MCMULLEN: *University of Alberta, Edmonton, AB, Canada, Canada*
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Microbiological sciences have advanced greatly over the years with many significant discoveries. However, microbes have continued to be major threats to public health and food safety. There is a disconnect between the amount of generated new knowledge on these pathogens to the amount of new and or improved interventions against them. A translational approach is therefore required to take food safety and public health to the next level. This round-table session aims to bring a global perspective on why some old pathogens such as *Listeria monocytogenes*, *Escherichia coli*, *Salmonella*, Norovirus, other viruses, and fungi have remained a challenge regardless of advancements in our knowledge about them. During this session, experts from academia, regulatory bodies, and industry will highlight the challenges of translating laboratory findings into practical real-life application and ways to overcome these hindrances.

Preliminary discussion points will focus on: (i) Summarizing the reasons why microbes remain a significant challenge in face of improved knowledge about them. (ii) Describing translational research and how to incorporate a translational approach in research and experimental design. (iii) Identifying advancements and challenges in the translation of laboratory findings to practical real-world application. (iv) Discussing areas where translational research is most critical and lacking. (v) Discussing how to bring about a change in research focus to have a more application approach and identify some of the gaps and concerns to be addressed.

Overall, we hope that this session will identify advancements and challenges to translating laboratory findings to real-life application and stimulate discussion to bring about a change in research focus.

RT19 Practical and Effective Approaches and Uses of Data in Retail and Foodservice Food Safety Programs

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MELANIE HARRIS: *Casey's General Stores, Ankeny, IA, USA*
MEGHANN MCLEOD: *Yum! Brands, Plano, TX, USA*
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BRANDON VOGA: *Big Y Foods, Springfield, MA, USA*
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The food safety industry has analyzed data sets for decades, but the industry has been on an accelerated crash-course in dashboards and insights over the last 4-5 years. New tech-enabled tools, understanding data plans, layering data sets, and assembling trends over time has really put us on a steep learning curve. No matter where you are in your data journey, this session will allow you an opportunity to hear what others have learned along the way.

So where are we today as an industry? Do you have "messy data"? Do you have lots of data, but are not able to harvest actionable insights? Are your data streams aligned by a data plan to allow for correlations and critical insights? Do you have access to food safety data analysts? Are you able to effectively and easily communicate your insights organizationally? Do you know where your highest environmental risk locations are?

This roundtable will focus on knowledge sharing of data journey success stories from food safety industry leaders in the retail grocery, convenience store, and foodservice sectors where the use of data drives budgeting, culture, and food safety program improvements. The panel will discuss the use of various data sets, such as electronic operational checklist data, audit data, health inspection data, internal data streams, and more. Join us and listen to how food safety leaders and their businesses have organized data architecture to provide key leadership metrics, align risk metrics to allocate focus and resources on their highest risk locations for action pans, provide communication to leadership on key benchmarks around handwashing compliance, and drive to improve food safety outcomes within their organizations. This knowledge sharing event to help spark ideas for you to take back to the office and utilize within your company.

RT20 Is Cultural Confirmation of Pathogens Obsolete?

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ERIC BROWN: *U.S. Food and Drug Administration – Center for Food Safety and Applied Nutrition, College Park, MD, USA*
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This roundtable is a forward looking sequel to the roundtable session from 2022 titled Rapid Methods and Automation in Food Microbiology: 40 Years of Developments, Promises, and Disappointments. Traditional culture, immunoassay, PCR and other genomic approaches have all required isolation of pure cultures for confirmation. Culture Independent Detection Technologies (CIDTs) such as BioFire® PCR array panels, and the targeted metagenomics that detect pathogens without enrichment or cultural confirmation are being increasingly used in clinical microbiology. The need for speed to detection and characterization of pathogens in the food industry is leading to advances in technologies that can detect, sero-type, strain type and sequence multiple pathogens in enrichment cultures. The speakers in this roundtable will share their experience while discussing the challenges, opportunities, and regulatory considerations with developing and implementing these technologies for rapid detection in food safety and spoilage arena.

RT21 Food Safety Extension Efforts for Small-Scale Urban Agriculture

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Urban agriculture, according to USDA, refers to the cultivation, processing, and distribution of agricultural products in urban and suburban settings, including warehouse farms, community gardens, rooftop farms, hydroponic and aquaponic facilities, and other innovative ways to grow different agricultural products. Urban farmers and gardeners collaborate and develop connections within diverse populations intending to expand access to nutritious foods, foster community engagement, connect with youth, provide jobs, educate communities about farming, and expand green spaces. Much of their produce may be sold direct-to-consumer or to restaurants and may not be covered by the Food Safety Modernization Act's (FSMA) Produce Safety Rule (PSR).

Oftentimes low-income, ethnically and racially diverse communities are both the intended consumers and the workforce in these urban agriculture initiatives. Engaging with communities participating in urban agriculture has great extension and outreach potential because of the emphasis and focus on community-building and resiliency. Supporting urban farming expansion supports the local food movement, focused on providing healthier food, reducing transportation costs while improving the food quality, making culturally-relevant food accessible, available, and affordable. Urban production environments also contribute to neighborhood beautification and sustainability. Nonetheless, this emerging farm sector presents unique hazards and thus provides a niche for outreach and engagement with growers, especially those outside of the PSR's scope. The goal is to ensure the food safety of these urban-farmed products.

The panelists are educators and other food safety professionals already working with urban growers and community members to implement Good Agricultural Practices (GAPs) and comply with PSR requirements within their operations. The goal of this panel is to discuss actionable areas that educators and other stakeholders can use to improve food safety practices by underserved, urban growers. The panel can discuss opportunities, successful approaches to engagement, and understanding barriers to connecting and implementing best practices within these spaces. The panelists will address the importance of GAPs and the intersection with regulatory requirements for urban farming in local food systems and their role in engaging urban growers.

RT22 Ensuring Food Safety within Global Supply Chains: Shared Learnings from Global Food Safety Enforcement Agencies and Educators

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CONRAD CHOINIERE: *Office of Analytics and Outreach, Food and Drug Administration, U.S. Department of Health and Human Services, College Park, MD, USA*
CAMERON PRINCE: *The Acheson Group, Ottawa, ON, Canada, Canada*
JERRY WOJTALA: *International Food Protection Training Institute, Portage, MI, USA*
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Food safety regulatory agencies around the world are responsible for public health by ensuring safe production and consumption of food within local, regional, national, and international supply chains. While agencies carry out inspections and enforcement to ensure food safety, their practices are guided

by regulations designed by national and international legislators. In 2020 we explored the challenges faced by regulatory agencies in performing their inspection and enforcement duties with the aim of developing a globally applicable assessment system which would aid agencies across the world assess and compare their performance towards achieving global food safety. As of 2022, we have identified the building blocks of a mature food safety regulatory agency and developed a framework to capture the performance of agencies which have been shared with some key stakeholders. The panel, comprising of these stakeholders, will share their perspectives on the challenges faced by various international agencies in maximising their performance to ensure food safety.

RT23 Overcoming Obstacles: How LGBTIQIA+ Individuals Can Thrive in the Field of Food Safety

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JOHN BERES: *Whole Foods, Orlando, FL, USA*
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Over the last several decades, the percentage of adults in the U.S. and globally that identify as a member of the LGBTQIA + community has increased. Despite the increasing visibility and openness of the LGBTQIA + community, a 2020 study from the Center for American Progress found that many LGBTQIA + individuals faced barriers and discrimination, including in their professional lives. For example, 35% of surveyed individuals reported that anti-LGBTQIA + discrimination limited or inhibited their ability to be hired, and 31% of individuals reported that their career progression was limited because they were a member of the LGBTQIA + community. Large numbers of respondents also chose their field and place of employment to avoid discrimination and bias. As a professional organization with a emphasis on mentorship and professional development, IAFP needs to champion diversity, equity, and inclusivity in support its diverse membership, including members of the LGBTQIA + community. This roundtable will foster discussion between students, early-career professionals in the field of food safety, and established professionals representing academia, industry, and government about their personal, lived experiences and perspectives as members of both the LGBTQIA + and the IAFP communities. This roundtable is one way for IAFP to publicly discuss and establish its support for its LGBTQIA + membership, highlight and signal to prospective students, developing professionals, and other IAFP membership that there are established IAFP members who are also a part of the LGBTQIA + community, who they can reach out to for mentorship, and to generate a sense of community and inclusivity in the global food safety community. The combined personal, lived experiences of the selected speakers include representatives from industry, academia, and government, at each career stage. Each panelist will not only discuss their personal, lived experiences, but will also provide insight into the food safety workforce, discuss how to attract and maintain a diverse workforce, and answer questions posed by the roundtable attendees.

RT24 From Bench-Top to Scale Up: The Unspoken Food Safety Challenges of Research and Development

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AMIT MOREY: *Auburn University, Auburn, AL, USA*
WENDY MADUFF: *Wonderful Company, Los Angeles, CA, USA*
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SHAWN STEVENS: *Food Industry Counsel, LLC, Milwaukee, WI, USA*

From chicken and waffle flavored potato chips to green ketchup, grocery store isles are filled with food products that come in more shapes, sizes, and flavors than you could ever imagine. Starting as just a concept, new products go through a seemingly straight forward yet challenging product development (PD) process from bench-top to pilot testing to final commercial launch with a lot of moving parts. Also, innovative products, new processing technologies, complex governmental regulations, and consumer demand for products free of preservatives and chemicals, has made developing new products increasingly challenging from a food safety perspective. Although food safety is an integral part of the process, it's often overlooked in these initial developmental stages due several reasons including the lack of food safety understanding among the product development scientists, a disconnect between PD and QA departments, and ambiguity in the regulations regarding new ingredients, etc. This roundtable will discuss the unique challenges faced by food scientists and manufactures in product development touching on the food safety and quality challenges when up scaling from initial concept to full production, how risks are managed and how decisions are made in regards to new ingredient selection, formulation, and new processes. This roundtable panel encompasses professionals from all stages of the development process and includes manufacturing PD, food safety, retail, food law, and government to discuss the many food safety factors and challenges that persist in transforming an idea into something safe and delicious.

Potential discussion points:

- Industry – When/how are food safety considerations introduced into the R&D process?
- Government – Does FDA give industry any guidance on food safety considerations for new product development?
- University – What are some of the most common problems that food companies face during the R&D process and how are you helping them scale up?
- Everyone – What are the biggest food safety challenges associated with new product development?

Roundtable

Technical Abstracts

T1-01 Growth of Salmonella during Preparation of a Fermented Cashew Cheese Analog

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❖ ***Developing Scientist Entrant***

Introduction: In the past decade, three salmonellosis outbreaks in Canada and the U.S. were linked to cashew "cheese" products; the behavior of *Salmonella* during plant-based fermentations is poorly understood.
Purpose: To evaluate the behavior of *Salmonella* during fermentation of cashews.
Methods: Cashews were soaked 1:1 w/v in water for 24 h at 4°C, drained and blended (1 min) with water and 0.2 g of commercial starter culture (Danisco, Choozit MM 100). Cashews with added starter culture (LAB), and rifampin-resistant *Salmonella* Enteritidis PT 30–inoculated cashews (1 log CFU/g) with or without starter culture (SAL+LAB or SAL, respectively) were held at 25°C for 72 h. Using standard methods, *Salmonella* levels, aerobic plate count (APC), and pH were measured in duplicate at 0, 24, 48, and 72 h in replicate experiments (n=4).
Results: Initial APC of 8.14±0.21 (LAB), 8.21±0.14 (SAL+LAB), and 1.96±0.08 log CFU/g (SAL) increased to maximum populations of ~9 log CFU/g by 24 h (LAB and SAL+LAB) or 48 h (SAL). The pH decreased significantly from initial pH 6.14±0.19 to 4.85±0.06 (LAB) and 4.87±0.10 (SAL+LAB) at 24 h, and 4.91±0.18 at 72 h (SAL). *Salmonella* levels increased significantly ($P<0.05$) by 1.29 log CFU/g from 1.17±0.36 to 2.81±0.18 and 2.46±0.02 log CFU/g after 24 and 72 h, respectively (SAL+LAB), or by 6.96 log CFU/g from 1.44±0.20 to 7.10±0.21 and 8.40±0.34 log CFU/g after 24 and 72 h, respectively (SAL).
Significance: Adding a starter culture reduced but did not prevent growth of *Salmonella*, indicating that additional control measures may be needed for plant-based fermented products.

T1-02 Efficacy of Ultra-Fine Ozone Bubbles in Inactivating *Listeria monocytogenes* on Fresh Produce

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❖ ***Developing Scientist Entrant***

Introduction: Wash water used for fresh produce can act as a source of contamination due to the widespread distribution of food borne pathogens in the environment. Commercial disinfectants are not completely effective in killing pathogens on the surface of produce.
Purpose: The overall goal of this project was to develop novel washing treatments using ultra-fine Ozone (UFO) bubbles to reduce the survival of *Listeria monocytogenes* on fresh produce (lettuce, celery and apples). In addition, the effect of UFO bubbles on color of aforementioned produce was tested using Miniscan® XE.
Methods: UFO bubbles were produced using an ozone injected-nanobubble generator system. The generated ozone nanobubble solution was characterized for size, concentration and oxidation potential. Thereafter, the antimicrobial efficacy of washing (for 1, 3, or 5 min at 25 or 4°C) with water containing UFO bubbles against *L. monocytogenes* on fresh produce was investigated. Experiments had triplicate samples, repeated twice, and analyzed using ANOVA.
Results: Bubble characterization results indicated that the bubble number in water was approximately 10^8/ml with size < 200 nm. The dissolved ozone concentration in UFO bubble water was ~5 ppm at 25°C. Washing of fresh produce with ultra-fine ozone bubble water significantly reduced *L. monocytogenes* load by ~1 to 1.5 log CFU/sample, as early as 1 min of treatment time ($P<0.05$). No significant increase in efficacy against *L. monocytogenes* on fresh produce was observed by increasing the wash time to 5 min ($P>0.05$). The wash treatment did not affect the color parameters (L, a, b values) of the fresh produce ($P>0.05$). No *L. monocytogenes* was detected in wash water (> 5 log CFU/ml reduction). Experiments investigating the efficacy of UFO bubble water in reducing the survival of *S. enterica* and *E. coli* O157:H7 on fresh produce are currently underway.
Significance: UFO bubble water could be used for produce decontamination without affecting the color of the product.

T1-03 Application of Lactic Acid Bacteria Against Shiga-Toxigenic *Escherichia coli* during Flume Washing of Leafy Greens

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❖ ***Developing Scientist Entrant***

Introduction: In the last two decades, leafy greens contaminated with Shiga toxigenic *Escherhicia coli* (STEC) have resulted in numerous outbreaks and recalls. STEC infection in humans could lead to severe health implications: kidney-failure, gastrointestinal-disorders and even death. These could also result in huge economic losses to the food industry. Lactic acid bacteria (LAB) are well-known for their probiotic properties, however, studies to evaluate their potential as antimicrobials, during flume washing of leafy greens, are limited.
Purpose: Evaluate LAB against STEC on spinach and romaine.
Methods: Two strains of LAB: *Lactobacillus acidophilus* C28 (LAC28) and *L. reuteri* X-18 (LRX-18) were evaluated against STEC O157, O26, O45, O103, O121, O145, and O111 on spinach and romaine. Leafy green pieces (approx. 2.5 by 2.5 cm; 1g) were prepared by washing with sterile distilled water and drying (30 min.). Prepared greens were inoculated with pathogen cocktails (5 log CFU/ml) and pathogen attachment allowed for 1 h. Leafy greens were then washed (2 min) with LAB treatments (8 log CFU/ml) or PBS (control) and stored (4°C) for three days. Surviving bacterial populations were enumerated on days 0, 1, and 3 by plating on appropriate medium. Data were analyzed using one-way ANOVA ($P<0.05$).
Results: On days 0 and 1, pathogen populations on romaine lettuce were reduced by 0.5 and 1 log after treatment with LRX-18 and LAC28, respectively. On spinach, ~0.8 log reduction was observed with both LAB treatments. On day 3, no noticeable reduction was observed with LRX-18 treatment, but leafy greens treated with LAC28 showed further reduction (~0.3 log) in STEC populations. Results indicate that both LAB treatments were more effective on romaine lettuce than on spinach with LAC28 performing better than LRX-18.
Significance: Lactic acid bacteria could be utilized as biocontrol agents in wash water for leafy greens to reduce STEC populations.

T1-04 Efficacy of Lactic Acid Bacteria as Wash-Water Treatments of Leafy Greens Contaminated with *Salmonella enterica* Typhimurium

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❖ ***Developing Scientist Entrant***

Introduction: Consumption of minimally processed leafy greens has increased in the past decade. This has been accompanied with increased food-borne illness outbreaks and recalls, with *Salmonella enterica* as one of the main culprits. Lactic acid bacteria (LAB) have been evaluated for their inhibition

Technical

capabilities against foodborne pathogens. However, limited studies have been conducted on their application as wash-water treatments, to reduce *Salmonella* contamination on leafy-greens.

Purpose: Determine the efficacy of LAB as wash-water treatments of spinach and romaine lettuce contaminated with *S.* Typhimurium.

Methods: *Lactobacillus acidophilus* C28 (LAC28) and *L. reuteri* X-18 (LRX-18) were used as wash-water treatments against *S.* Typhimurium DT104 (H2662) on spinach and romaine. Leafy greens (approx. 2.5 by 2.5 cm pieces; 1g) were prepared by washing with sterile distilled water and drying (30 min.). Prepared leafy greens were inoculated with *Salmonella* (5 log CFU/ml), and pathogen attachment allowed for 1 h. Following inoculation and attachment, spinach and romaine were washed (2 min) with LAB (8 log CFU/ml) or PBS (control) treatments and stored at 4°C for 3 days. Surviving *Salmonella* populations were determined on days 0, 1, and 3, by plating on XLD agar. Data were analyzed using one-way ANOVA ($P<0.05$).

Results: Salmonella Typhimurium populations on romaine lettuce were immediately (day 0) reduced by 0.8 and 0.5 log when treated with LAC28 and LRX-18, respectively. This reduction was maintained on romaine treated with LAC28, whereas that treated with LRX-18 showed further reduction of ~1 log. On spinach treated with LAC28, a reduction of ~0.6 log was observed on day 3, while an immediate (day 0) reduction in *Salmonella* population was observed on spinach treated with LRX-18, which was maintained over three days. Both LAB treatments were able to reduce pathogen populations on the leafy greens with better results on romaine lettuce than on spinach

Significance: Lactic acid bacteria could be used to control *Salmonella* Typhimurium on leafy greens.

T1-05 Simulation of Elimination and Contamination of *Escherichia coli*, *Listeria monocytogenes,* and MNV-1 from the Wash Process When Handling of Potatoes

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Introduction: Root vegetables, which are in close contact with soil, are particularly vulnerable to soil contamination or decay as they can be contaminated from multiple sources including primary production and processing.

Purpose: This study investigated effective washing conditions to reduce the microbial contamination of potatoes using soaking and shaking in the washing process.

Methods: The reduction of *Escherichia coli, Listeria monocytogenes*, and murine norovirus (MNV-1) in four washing treatments (soaking only, shaking only, soaking/shaking, and soaking/shaking/shaking) were compared.

Results: At washing treatment, The MNV-1 titer of potatoes decreased by 1.22 to 1.42 log GC/100 g when soaked in water. The combined treatment reduced the microbial contamination more efficiently than shaking only. The reduction rate of *E. coli* in the washing process was higher than for *L. monocytogenes*. The reduction of MNV-1 was similar regardless of shaking or combination treatment.

Significance: Combined treatment (soaking-shaking-shaking) is an appropriate method to reduce all pathogens in washing water. These results can be used as basic data for scale-up research on the cleaning process of agricultural products.

T1-06 Cross-Transfer of Foodborne Pathogens during Peach Hydrocooling

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Introduction: Sanitizers are commonly used to reduce cross-contamination during the post-harvest hydrocooling of produce.

Purpose: The purpose of this study was to evaluate the efficacy of chlorine is reducing transfer of *Escherichia coli* TVS353 and *Listeria monocytogenes* during the hydrocooling of peaches.

Methods: Four rows consisting of 3 peaches/row (n=12) were stacked in two mesh baskets. The top row of peaches was seeded with rifampicin-resistant *E. coli* TVS353 (9.6404 log CFU/ml) or a five-strain cocktail of *L. monocytogenes* (9.3401 log CFU/ml) and allowed to dry for 2 h to facilitate attachment. The population of *E. coli* TVS353 and *L. monocytogenes* at the start of hydrocooling was 6.110 and 6.0502 log CFU/g peach, respectively. Peaches were cooled by pumping (1.4 L/min) chlorinated water (80 ppm free chlorine, 3.2516°C) on to the baskets through a shower head for 1 minute. Sterile deionized water (SDW) was used to hydrocool the peaches as a comparative control. All peaches and cooling water were aseptically collected for quantification of transfer of *E. coli* TVS353 and *L. monocytogenes* and for three biological and technical replicates. Significant differences were determined using ANOVA.

Results: Hydrocooling using 80 ppm chlorine resulted in a significant reduction in row 1 of peaches by 0.1329 and 1.81.59 log CFU/ml for *E. coli* TVS353 and *L. monocytogenes*, respectively ($P<0.05$). There was no transfer of *E. coli* TVS353 detected from row 1 downwards. The population of *L. monocytogenes* on rows 2, 3 and 4 was 1.298, 1.1063, and 0.999 log CFU/g peach, respectively. Transfer of *L. monocytogenes* among peaches during hydrocooling was significantly higher than *E. coli* TVS353.

Significance: Chlorination of water can significantly reduce cross transfer among peaches during hydrocooling. Future work will evaluate lower inoculum levels and other sanitizers used during hydrocooling.

T1-07 Consumers' Willingness to Pay for Produce with a Food Safety Label from Small- and Medium-Sized Farms

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Introduction: Small- and medium-sized farms (SMSF) make up most of the family farms in the U.S. and contribute slightly under half (about 42%) of the overall production value. Little is known whether the presence of food safety labels and farm size affect consumers' willingness to pay (WTP) for produce.

Purpose: To identify consumers' WTP for produce with a food safety label, and their WTP for produce from various sizes of farms.

Methods: We administered an online survey to consumers who purchase bell peppers, fresh kale, and/or fresh spinach. We divided the survey into eight sections, including a WTP section with four scenarios to purchase produce. Each scenario included farm size, label, and inspection status. We first applied a Kendall's tau correlation test to assess consumers' WTP across the scenarios. We then used a discriminant model to assess which variables affected consumers' differing views on their WTP.

Results: Out of the 914 consumers surveyed, 661 (72.3%) said they are WTP more for produce from SMSFs if the produce has a food safety label ($P<0.001$). Additionally, we found a positive correlation between consumers' WTP for produce with a food safety label from both SMSFs and large farms (r=0.681) ($P<0.001$). Based on the discriminant model ($P<0.001$), we found the following top predictors for consumers' WTP for produce with a food safety label from SMSFs: (1) consumers who live with adults over the age of 65 (β 0.64); (2) those who blame government agencies for outbreaks (β 0.63); and (3) those who characterize produce purchased from SMSFs rather than large-sized farms as helping to sustain local farms (β -0.831).

Significance: These findings shed light on consumers' decision-making process of produce origin and food safety labels and can support future food safety policy and education program development for SMSFs.

T1-08 Population Dynamics of *E. coli*, *Listeria*, and *Salmonella* on Fresh Produce: A Scoping Review

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❖ ***Developing Scientist Entrant***

Introduction: Collation of the current scope of literature related to population dynamics (i.e., growth, die-off, survival) of foodborne pathogens on fresh produce can aid in informing future research directions and help stakeholders identify relevant research literature.

Purpose: A scoping review was conducted to gather and synthesize literature relevant to the research question, "What is the available information and how complete is the information on population dynamics of *Escherichia coli*, *Listeria* spp., and *Salmonella* spp. on unprocessed fresh produce?" Unprocessed fresh produce is defined here as produce not having undergone chopping, cutting, homogenization, irradiation, or pasteurization.

Methods: Literature studies were identified in a comprehensive database search (e.g., PubMed, Web of Science, ProQuest) of records published prior to September 23, 2021, followed by screening for relevance based on eligibility criteria. Studies (i) available in English that (ii) investigated population dynamics of *E. coli*, *Listeria* spp., and/or *Salmonella* spp. on (iii) at least one unprocessed fresh produce commodity, such that (iv) no antimicrobial agents were applied to produce following inoculation or contamination, were considered eligible for inclusion.

Results: Of the 16,502 records screened, 676 underwent full-text review, and of those a total of 277 studies met all eligibility criteria and were subjected to an in-depth qualitative review of key study characteristics (e.g., produce commodities evaluated, cultivation practices used, simulated storage conditions, inoculation methodologies). Included studies represented investigations of population dynamics on produce before (i.e., pre-harvest; n=143) and after (i.e., post-harvest; n=144) harvest. Pre-harvest studies investigating population dynamics of *Listeria* spp. (n=13) were notably underrepresented compared to *Salmonella* spp. (n=69) and *E. coli* (n=93). Additionally, a low frequency of pre-harvest studies reported evaluating microbial population dynamics on produce grown using hydroponic cultivation practices (n=7).

Significance: These and other knowledge gaps identified in this scoping review represent areas of research that can be further investigated in future studies.

T1-09 Exposure of Canadians to the Food Safety Risks in Fresh Fruits and Vegetables Evaluated through a National Consumption Survey

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Introduction: When assessing the Canadian microbial health burden associated with food commodities, fresh fruits and vegetables (FFV) represents the second highest after meat. This information along with other risk factors are used by the Canadian Food Inspection Agency's Establishment-based Risk Assessment (ERA)-Food model to evaluate the food safety risk of regulated establishments and allocate inspection resources.

Purpose: Estimate the risk exposure associated to FFV, by quantifying the sub-products' annual volume consumed by the Canadian adult population.

Methods: An online survey was designed to collect FFV consumption in the past 24 hours prepared at home, by recruiting participants using a proprietary interactive voice response and in-house computer-assisted telephone interviewing calls. The survey comprised multiple-choice demographic questions, and participants were asked to indicate the number of servings consumed and the state of the FFV item (fresh intact or minimally processed). Data were collected throughout the four seasons (one full year). Soft quotas were applied to ensure sampling was representative of the Canadian population. Selection frequencies, the average number of servings consumed, standard error of the mean, and the 95th percentile were calculated for all sub-products, making a distinction based on the item state before consumption.

Results: Over 9,000 surveys were analysed with the proportion of participants being representative of each Canadian province population. About 90% of participants were between the ages of 27 and 76, covering three generations equally. Consumption patterns were collected for seven categories and 188 FFV sub-categories, highlighting trends like lettuce being the most commonly leafy vegetable consumed (0.30 selection frequency), showing the highest consumption in the summer (0.67±0.03 servings/day) followed by spinach (0.30±0.04 servings/day) and baby salad greens (0.18±0.02 servings/day).

Significance: These findings provided insight into Canadian consumption patterns specific to FFV and were incorporated into the ERA-Food model to accurately assess food safety risk.

T1-10 International Foodborne Outbreaks Attributed to Leafy Green Vegetables, 2000-2021: An Overview and Descriptive Analysis

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Introduction: The increased availability and production of leafy green vegetables in many convenient forms has resulted in higher numbers of outbreaks associated with these products over the last two decades.

Purpose: The objective of this report is to examine the characteristics of global foodborne outbreaks attributed to leafy green vegetables from 2000-2021, providing an overview and insights into their occurrence including the trends and circumstances under which they occur.

Methods: The data for this analysis was extracted from the Public Health Agency of Canada's Publicly Available International Foodborne Outbreak Database (PAIFOD); a repository of international foodborne outbreak data systematically collected from various sources since 2000. To identify potential leafy green vegetable outbreaks, a query was run within PAIFOD. Descriptive analysis of outbreak impacts, vehicles and microorganisms, geography, settings, causative reason, and temporality was performed using Microsoft Excel 2016.

Results: From 2000 thru 2021, 616 outbreaks were identified attributed to either leafy green vegetables (n=237) or leafy green vegetable-based salads (n=379). Leafy green vegetable outbreaks showed an increasing trend in occurrence and were frequently attributed to highly pathogenic bacteria such as *Escherichia coli* (42%). The most commonly implicated food vehicles were unspecified lettuce (38%), romaine lettuce (18%) and leafy greens (17%). The causative reason was frequently related to contamination at the farming or processing level leading to widespread outbreaks spread through mass distribution chains. Leafy green vegetable-based salad outbreaks have been stable and were frequently caused by norovirus (67%). The causative reasons for leafy green vegetable-based salad outbreaks were mostly related to errors in food safety procedures during food preparation.

Significance: A wide range of pathogens were associated with leafy green vegetables, some were common (STEC, *Salmonella* and norovirus) and others were only recently associated with leafy green vegetables, underscoring the challenges in mitigating food safety risks along the farm to fork continuum.

T1-11 Transfer Level of Shiga Toxin-Producing *Escherichia coli* O157:H7*, Salmonella enterica* and *Listeria monocytogenes* from Growing Media and Seeds to Microgreens

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Introduction: Microgreens are considered as immature seedlings of different type of crops that can be contaminated by various preharvest sources including growth media, irrigation water, and seeds.

Purpose: The aim of this study was to determine the transfer level of Shiga toxin-producing *Escherichia coli* O157:H7 (STEC), *Salmonella enterica* and *Listeria monocytogenes* to the edible part of various type of microgreens from plant growth media-soaked perlite as growth media and seeds.

Methods: Ampicillin (Amp) resistant three-strain cocktails of STEC and *Salmonella* and non-resistant *L. monocytogenes* were inoculated into plant growth media-soaked perlite as direct soil substitute in low (10^2 to 10^3 CFU/g) and high (10^5 to 10^6 CFU/g) populations. Twenty species of microgreens were grown in

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inoculated perlite. Also, seeds of five microgreens were inoculated with low and high population of pathogens in similar concentrations before planting. After harvest, concentration of pathogens transferred to the edible part of microgreens from contaminated perlite and seeds were enumerated on selective media. SMAC and XLD supplemented with Amp (100 µg/ml) was used for STEC and *Salmonella*. PALCAM was preferred for *L. monocytogenes*.

Results: Pathogen populations transferred to microgreens depended on the pathogen and microgreen type in general. Contamination source and inoculation level did not affect the transfer level constantly due to rapid population increase of pathogens in growth media. Transfer level of pathogens from contaminated perlite to microgreens ranged from 1.62±0.18 log CFU/g (the lowest: *Salmonella*, gin pepper) to 7.23±0.15 log CFU/g (the highest: STEC O157:H7, radish) ($P<0.05$). Pathogen populations transferred to microgreens from inoculated seeds grown were between from 2.70±0.15 log CFU/g (the lowest: *L. monocytogenes*, pea) and 7.74±0.07 log CFU/g (the highest: STEC, mustard) ($P<0.05$).

Significance: Plant growth media-soaked perlite as direct soil substitute and seeds can be source of microgreen contamination when introduced with *S. enterica*, STEC O157:H7 and *L. monocytogenes* regardless of inoculation level and type of microgreens.

T1-12 Inactivation of Foodborne Pathogens on Apples through Application of Antimicrobial Waxes

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Introduction: There is a need for effective postharvest controls capable of effectively inactivating foodborne pathogens which can be applied to organic as well as conventional fruit.

Purpose: To evaluate the efficacy of natural antimicrobials (essential oils or cultured dextrose) when incorporated into carnauba wax and applied to organic apples.

Methods: Organic Gala apples were spot inoculated with five strains/serovars cocktail (~9 log CFU/ml) of *Listeria monocytogenes, Salmonella*, or Shiga-toxigenic *E. Coli* (STEC). Inoculated apples were coated with organic carnauba wax containing a 1% and 2% (v/v) mixture of essential oils in equal proportions (oregano, clove bud, cinnamon bark, and coriander) or commercially cultured dextrose at 0.5% (w/v). Apples coated with carnauba wax only and uncoated apples were used as control treatments. Apples were stored at 20°C and sampled for up to 40 days. Five treatments with three biological replicates and three samples per replicate were used for each treatment (n=9).

Results: Carnauba wax containing 2% essential oils was the most effective for inactivating target foodborne pathogens inoculated on apples during 40-day storage at 20°C. This treatment showed 4.03, 2.81, and 1.41 log CFU/apple reduction in *L. monocytogenes*, *Salmonella*, and STEC, respectively, compared to uncoated apples. At the end of the storage period, carnauba wax containing 2% essential oils showed significantly ($P<0.05$) lower populations of *L. monocytogenes* and *Salmonella* compared with 1% essential oils, 0.5% cultured dextrose, or wax-only control. In contrast, there were no significant differences ($P>0.05$) for STEC.

Significance: This study provides information to the organic apple industry about the efficacy of antimicrobial waxes to inactivate bacterial foodborne pathogens. Organic carnauba wax containing 2% essential oils was shown to be significantly more effective than wax-only control; Subsequent research should evaluate organoleptic and quality attributes to assess fitness for application by the apple industry.

T2-01 Cesin, a Short Natural Variant of Nisin, Displays Potent Antimicrobial Activity Against Major Foodborne Pathogens Despite Lacking Two C-Terminal Macrocycles Essential for Bioactivity of Full Length Nisin

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❖ ***Developing Scientist Entrant***

Introduction: Nisin is a widely used lantibiotic owing to its potent antimicrobial activity and its food-grade status. Its mode of action includes cell wall synthesis inhibition and pore formation, which are attributed to the lipid II binding and pore forming domains, respectively. We discovered cesin, a short natural variant of nisin, produced by the psychrophilic anaerobe *Clostridium estertheticum*. Unlike other natural nisin variants, cesin lacks the two terminal macrocycles constituting the pore forming domain. The influence of this feature on the potential of cesin a novel food bio preservative is unknown.

Purpose: The current study aimed at elucidating the effect of lacking the essential pore forming domain on the antimicrobial activity of cesin.

Methods: Cesin was heterologously expressed cesin in *Lactococcus lactis*, and its antimicrobial activity determined using minimum inhibitory concentrations and killing assays. Its mode of action was determined using lipid II and lipoteichoic binding assays and fluorescence microscopy. Stability against different pH, temperature and enzyme conditions were determined. Comparisons were made against nisin. The functionalities of cesin were improved through bioengineering.

Results: Cesin demonstrated a broad and potent antimicrobial profile comparable to that of nisin. Its mode of action was linked to lipid II binding and electrostatic interactions with teichoic acids. Fluorescence microscopy showed that cesin lacks pore forming ability in its natural form. Stability tests have shown the lantibiotic is highly stable at different pH values and temperature conditions, but that it can be degraded by trypsin. However, a bioengineered analogue, cesin R15G, overcame the trypsin degradation, while keeping full antimicrobial activity.

Significance: This study shows that cesin is a novel (small) nisin variant that efficiently kills target bacteria by inhibiting cell wall synthesis without pore formation. Cesin can therefore be developed as a food biopreservative.

T2-02 Characterization and Application of a Novel, Cold-Robust Phage for Control of *Salmonella* and Its Biofilm on Cantaloupe Under Cold Temperature

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Introduction: The exposure of cold temperatures for cantaloupe during its washing and storage is inevitable. The stable and persistent efficacy of phages under various temperatures depends on the host's physiological status, which should be ensured for practical and reliable usage.

Purpose: The purpose of this study was to characterize a novel *Salmonella* phage and its performance on antibiofilm efficacy at cold temperature.

Methods: The specificity and efficiency of plating (EOP) of the phage were evaluated over 10 *Salmonella* and 27 non-*Salmonella* strains at 4, 22, and 37 °C. Genetic analyses were performed to identify its safety and the presence of biofilm-degrading enzymes. Cantaloupe (2 by 2 cm) was submerged into the *S*. Typhimurium suspension (10^6 CFU/ml) and incubated for 48 h at 4 and 22 °C, mimicking its realistic conditions. After confirming the biofilm formation using SEM, the cantaloupe was dipped into the phage suspension with an MOI of 100 at both temperatures for 2 h. Antibiofilm efficacy of phage was assessed by XLD plate count method and SEM observation.

Results: The phage could specifically infect five *Salmonella* strains with a great efficiency (EOP>0.6) at all tested temperatures. Genetic analyses confirmed its safety and identified endolysin and two depolymerases in tail protein possessing antibiofilm potential. The initial biofilm population at 22 °C (~6.5 log CFU/cm^2) was significantly greater than that at 4 °C (~4.5 log CFU/cm^2) ($P<0.05$), implying the cold effect on *Salmonella*. After phage treatment, it reduced almost of the biofilm at 4 °C (~4.4 log CFU/cm^2), which was significantly greater than at 22 °C (~2.6 log CFU/cm^2) ($P<0.05$). SEM observation also revealed the more effective destruction of cohesive and dense biofilm architecture on cantaloupe at 4 °C.

Significance: This study demonstrated the practical performance of the phage for controlling *Salmonella* and its biofilm on fresh produce under simulated cold condition.

T2-03 Expression of Broad-Spectrum Endolysin-1252 in Controlling Multiple Serovars of Salmonella enterica

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* is the most common foodborne pathogen worldwide that causes a large number of hospitalizations, death as well as significant economic loss. Further, due to the overuse of antibiotics, the incidence of multiple antibiotic-resistant *S. enterica* has been growing rapidly, which warns as a serious healthcare crisis, specifically the shrinking effects of antibiotic therapeutic options. Recent studies show that endolysin from bacteriophages could be a promising alternative to replace antibiotics and/or control colonization of *S. enterica* in the reservoir.

Purpose: To generate a recombinant beneficial bacterial strain by expressing endolysin, which was isolated from novel bacteriophage-1252 that showed a lytic effect against multiple serovars of *S. enterica*, specifically Typhimurium and Enteritidis.

Methods: A novel bacteriophage-1252 was isolated, sequenced, and annotated to identify genes encoding for endolysin named Endolysin-1252. Endolysin-1252 was predicted to possess transglycosylase activity with 240 amino acid residue sequence. His-tagged recombinant endolysin-1252 was encoded and amplified by lytic *Salmonella* bacteriophage-1252 and was obtained by prokaryotic expression using quantitative PCR measuring. Finally, endolysin-1252 was purified by Ni-NTA chromatography and characterized its phenotypic characteristics and lytic ability against various serovars of *S. enterica*.

Results: A broad-spectrum endolysin-1252 was successfully expressed in BL21 (DE3) *Escherichia coli*. The qRT-PCR assays confirmed the expression (1.6 folds) of this gene. Using Ni-NTA chromatography, we also verified the molecular weight of Ni-NTA chromatography of the endolysin. Biological effects, including growth inhibitory effectiveness against multiple serovars of *S. enterica* and reduction of their interaction (adherence and invasion) with host (INT-407) cells, of this endolysin showed promising outcomes in controlling multiple serovars of *S. enterica*.

Significance: These findings indicate that this broad-spectrum endolysin-1252 could be a promising alternative to conventional antibiotics in controlling the colonization of multiple serovars *S. enterica*.

T2-04 Antimicrobial Activity and Identification of Genome-Related Bacteriocin of *Lactobacillus gasseri* SMFM2021-S6 Isolated from Infant Feces

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Introduction: Antimicrobial activity of probioitcs appears through competitive intestinal adhesion with intestinal pathogens, or metabolites produced by the probiotics. *Lactobacillus gasseri,* one of the dominant species inhabiting in infant gut, was found to have antimicrobial activity against foodborne pathogens.

Purpose: The objective of this study was to evaluate the antimicrobial activity of *Lactobacillus gasseri* SMFM2021-S6 isolated from infant feces and to analyze its whole genome sequence.

Methods: *L. gasseri* SMFM2021-S6 was isolated from infant feces and identified with its 16s rRNA sequence. *L. gasseri* SMFM2021-S6 and *Lactobacillus rhamnosus* GG (positive control) were spot inoculated on de Man Rogosa agar at 8 Log CFU/mL, and they were incubated at 37°C for 24h. Two mixtures of foodborne pathogens (*Bacillus cereus strains* NCCP14043, NCCP14796, NCCP15909, NCCP15910 and NCCP16296, and *Escherichia coli* strains NCCP14043, NCCP14796, NCCP15909, NCCP15910 and NCCP16296) inoculated in brain heart infusion agar at 6 log CFU/ml were overlaid over the spots of *Lactobacillus* strains. The plates were incubated at 30°C for 24 h for *Bacillus cereus*, and 37°C for 24 h for *Escherichia coli* strains. The sizes of the clear zones were eventually measured. The whole genome sequence of *L. gasseri* SMFM2021-S6 were obtained and analyzed for the annotation.

Results: *L. gasseri* SMFM2021-S6 was new probiotic strain and had the high antimicrobial activities to *B. cereus* (7.71±1.29mm) and *E. coli* (10.08±1.53mm), which showed no significant difference with the positive control. In addition, *L. gasseri* SMFM2021-S6 genome has four areas of interest, which encode bacteriocins such as acidocin B, bacteriocin_LS2chainb, helveticin J, and gassericin T.

Significance: These results indicate that *L. gasseri* SMFM2021-S6 can be assumed to have antimicrobial activities, and the antimicrobial activities can be induced by bacteriocin productions.

T2-05 Evaluation of the Effect of Different Antimicrobials on the Quality and Shelf Life of Ready-to-Eat Hummus

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Introduction: The consumption of RTE food had significantly increased over the past years to meet a healthier and fast-paced lifestyle. Hummus is a very popular Mediterranean RTE food with growing popularity worldwide. However, hummus is susceptible to microbial contamination and microbial growth that limit its quality and shelf-life.

Purpose: For this purpose, the present study compared the use of several antimicrobials alone or in combination for hummus preservation.

Methods: The chemical preservative potassium sorbate 0.09% (S) was evaluated along with three natural antimicrobials: Garlic 1.25% (G); Vinegar 5% (V); Natamycin 0.002 % (N); or their combination: Garlic 1.25% –Vinegar 5% (GV); Vinegar 5% -Natamycin 0.002 % (VN); Garlic 1.25% -Natamycin 0.002 % (GN); and Garlic 1.25% -Vinegar 5% -Natamycin 0.002 % (GVN) to increase the shelf-life of hummus during storage (4˚C). Thymol and carvacrol mixture 0.2% (O) was also assessed to preserve and develop a new oregano-flavored hummus.

Results: All treatments that included vinegar used alone or in combination had significantly higher antimicrobial effectiveness against total aerobic counts (TAC), *Pseudomonas* spp., and lactic acid bacteria (LAB) than the other treatments. GVN combination showed the highest antimicrobial efficiency against the three former microorganisms. According to TAC, the shelf life of C, S, N, G, GN, and O was *ca.* 19 days, compared to an extended one of *ca.* 25 days for V and VN, and *ca.* 30 days for GV and GVN. Yeasts and molds were not detected in the treated samples. Sensory analysis showed the highest acceptability for C, N, S, V, and VN, followed by GV and GVN and the lowest one was for G, GN and finally O. The combination of garlic with vinegar improved the sensorial acceptability of treatments containing garlic.

Significance: The findings provide a potential natural method for hummus preservation and shelf-life extension.

T2-06 Application of Lactic Acid Bacteria Biofilms to Prevent or Remove *Salmonella enterica* Biofilms

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is a common human pathogen, causing multiple foodborne outbreaks and recalls. It can form biofilms on food-contact surfaces, allowing it to survive decontamination treatments. Development of antimicrobials that could inhibit these biofilms and the pathogen, are therefore needed. Lactic acid bacteria (LAB) are known to inhibit *Salmonella* spp., however, limited studies exist on the efficacy of LAB biofilms against *Salmonella* biofilms.

Purpose: Evaluate LAB biofilms to inhibit biofilms of *Salmonella enterica* Typhimurium DT104.

Methods: Using the agar-spot-test, 65 LAB strains were first evaluated for inhibition against four *S. enterica* Typhimurium DT104 (H2662, 152N17-1,

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13068A, 11942A) strains. Biofilms of LAB strains that showed excellent inhibition (zones>10 mm) were further tested against pathogenic biofilms. LAB ($1x10^8$ CFU/ml) were allowed to form biofilms in 96-well plates (48 h @ 37/42°C ; anaerobic incubation), before (preventive) or after (corrective) the formation of *S. enterica* biofilms. *Salmonella* strains were grown to achieve an OD (A_{600}) of ~$1x10^5$ CFU/ml prior to forming biofilms (48 h @ 37°C). Disruption of pathogen-biofilms was determined by measuring absorbance (A_{595}), while *S. enterica* populations were determined by enumerating on XLD-agar. Data were analyzed using one-way ANOVA ($P<0.05$).

Results: Of the 65 strains, 67% showed excellent (>15 mm), 25% very good (>10 mm), and 8% good (>5 mm) inhibition against *S. enterica* Typhimurium. In preventive treatments, *L. animalis* and *L. bulgaricus* biofilms were the most effective ($P<0.05$), reducing pathogen populations to undetectable levels (control=7.8 logs), followed by other LAB (2.8 to 6.2 logs reduction). In corrective treatments, majority (83%) of LAB biofilms reduced pathogen populations (control: 8.4 logs) to undetectable levels, while the other LAB biofilms achieved up to 6.4 logs reduction in *Salmonella* populations. The LAB biofilms were more effective in reducing *S. enterica* Typhimurium biofilms as corrective than preventive treatments.

Significance: Biofilms of LAB can be used effectively to reduce *S. enterica* Typhimurium and their biofilms.

T2-07 The Antibacterial Activity of Hemp (*Cannabis sativa* sp)Extract Embedded PVA Nanofibers Against *Listeria monocytogenes* and *Salmonella enterica* (spp) on Chicken Breast Meat

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Requested Changes:

Methods: In this study, Hemp extract was encapsulated into polyvinyl alcohol (PVA) electrospun fibers at different concentrations (0%, 5%, 7%, and 9% v/v) and electrospun with the Fluidnatek Electrospinning. The physicochemical properties of the electrospun nanofibers were characterized with FTIR. Antimicrobial activity was conducted after nanoencapsulation with hemp extract. Invitro assessment was conducted by broth tube immersion of the nanofibers and in-situ evaluation was conducted by wrapping 10g cuboids, SE inoculated (~10^7 CFU/mL), chicken tenderloin breast meat in the nanofiber and storing them at 4°C and 25°C. *4°C was used be*cause chicken is stored at this temperature during transport, storage, and home use and used to assess nanofiber performance under this temperature condition. 25°C was an accelerated condition to evaluate nanofiber antimicrobial performance under exaggerated temperature conditions. *All* treatments were analyzed in triplicate, and statistical significance was tested at 5%.

T2-08 Vapor Phase Antimicrobial Activity of Unencapsulated and Encapsulated Native Australian Essential Oils Against Foodborne Microbes

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❖ ***Developing Scientist Entrant***

Introduction: Food spoilage and pathogenic microorganisms are a persistent problem in the food industry, hence the need to explore alternative antimicrobial agents such as essential oils.

Purpose: Essential oils are volatiles, yet their vapor phase antimicrobial activity is underexplored; therefore, this study investigated the *in vitro* liquid and vapor phase antimicrobial activity of non-encapsulated and encapsulated Tasmanian mountain pepper (*Tasmannia lanceolata*) and lemon myrtle (*Backhousia citriodora)* essential oils against foodborne microbes.

Methods: The minimum inhibitory concentration of the essential oils in liquid and vapor was determined against a panel of eleven foodborne pathogenic and spoilage microbes using broth microdilution and vapor phase MIC assay. The chemical composition of liquid and vapor phases was studied using GC-MS and headspace Solid phase microextraction (SPME). Oils exhibiting potent antimicrobial activity in liquid and vapor phases were encapsulated in food-grade lipids to create nanostructured lipid carriers using high-pressure homogenization. Morphology, size, and stability of the encapsulated EO's were characterized using Cryo-Transmission electron microscopy, dynamic light scattering and turbiscanning respectively.

Results: In the liquid phase, the oils had comparable MICs (0.097- 0.390 % v/v) against Gram-positive bacteria, however, lemon myrtle was more effective against Gram-negative bacteria. In the vapor phase, lemon myrtle and Tasmanian mountain pepper inhibited nine and three out of the 11 microbes tested, respectively. Although the liquid phase activity was higher than the volatile phase, volatiles of the oils were more effective against the fungal species tested thus emphasizing their potential as alternative vapor phase antifungals. The major compounds in lemon myrtle were *Z*-citral and E-citral and polygodial in Tasmanian mountain pepper. The essential oil containing nanostructured lipid carriers were antimicrobial, < 200nm in size, showing stability at 2, 25 and 37 °C over 90 days.

Significance: Essential oils are promising alternative antimicrobial agents in both in liquid and vapour phases for food and food packaging applications.

T2-09 Nmca Carbapenemase-Producing *Enterobacter ludwigii* C1 from Carrots

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Introduction: Antibiotic resistance is an increasing challenge to public health globally. Carbapenems, which are considered the most effective beta-lactam antibiotics, are essential for the management of difficult-to-treat bacterial infections. Food products, including fresh vegetables, may serve as carriers for antibiotic-resistant bacteria.

Purpose: The purpose of the study was to characterize a carbapenem-resistant bacterium from a retail sample of carrots.

Methods: The strain was isolated from carrots using CHROMagar mSuperCARBA after an enrichment in buffered peptone water (BPW) and a selective enrichment in Enterobacteriaceae Enrichment Broth (EEB). Antibiotic resistance phenotype was tested using the standard disk diffusion method according to the Clinical and Laboratory Standards Institute protocols. The presence and the type of carbapenemase was tested using the mCIM/eCIM procedures. The whole genome of the new isolate was sequenced using Illumina NextSeq. After genome assembly, the species was identified by Ribosomal Multilocus Sequence Typing (rMLST). Multi-locus sequence typing (MLST) was carried out using the online PubMLST program. Antibiotic resistance genes were identified using the Resistance Gene Identifier (RGI) software and the Comprehensive Antibiotic Resistance Database (CARD).

Results: The new isolate C1 was resistant to multiple antibiotics, including ampicillin, amoxicillin-clavulanate, and imipenem. The strain showed positive mCIM and negative eCIM results, indicating the production of a serine-type carbapenemase. The isolate belongs to sequence type ST 748 and was identified as *Enterobacter ludwigii* C1. Genes encoding NmcA carbapenemase, AmpC beta-lactamase, fosfomycin resistance (FosA2), and antibiotic efflux pumps were identified in the genome.

Significance: *E. ludwigii* is of clinical relevance and has been isolated from patients. Antibiotic-resistant *E. ludwigii* may pose a potential risk to human health. To the best of our knowledge, this is the first report about the isolation and characterization of a carbapenem-resistant *E. ludwigii* from fresh vegetables.

T2-10 Poultry Industry-Wide Surveillance of Antimicrobial Use and Antimicrobial Resistance; Impacts of the Antimicrobial Use Reduction Strategy

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Introduction: The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) monitors antimicrobial use (AMU) and antimicrobial resistance (AMR) in broiler chicken and turkey flocks and conducted pilot surveillance in layer hens. These meat and egg producing poultry are essential to Canada's food system. Surveillance of AMU and AMR are critical to the safety of animals raised for human consumption in Canada and fulfills the national and global directives to contain AMR. In parallel, the poultry industry has implemented a voluntary policy to limit AMU to reduce AMR associated with poultry products.

Purpose: To assess AMR trends in *Salmonella, E. coli* and *Campylobacter* from farms and abattoirs (2017-2021) in relation to the industry-wide voluntary policy eliminating the prophylactic use of antimicrobials.

Methods: CIPARS collects AMU, farm-level demographic data and samples through a network of veterinarians and producers. Healthy chickens are also sampled at the abattoir to complement the farm-level results. Microbroth dilution was used to susceptibility test recovered bacteria. Descriptive statistics for AMU/AMR and animal health were generated.

Results: The percentage of flocks exposed to medically important antimicrobials decreased (Broilers: n=665 flocks, 82% to 66%, Turkeys: n=438, 83% to 29%). The quantity of antimicrobials (milligrams/kg animal biomass) decreased during the surveillance timeframe by 8% and 17% in broiler chickens and turkeys, respectively. Limited layer flocks (n=10/74) used antimicrobials. In parallel, various AMR measurements trended downwards, notably, the % resistance to ≥ 3 antimicrobial classes in *E. coli* (Broilers: 39% to 19%, Turkeys: 39% to 12%), and sustained low level resistance to third generation cephalosporin (<5% in both species). In layers, 70% of the isolates were susceptible to all antimicrobials in the panel.

Significance: Surveillance and monitoring of AMU and AMR are essential in monitoring AMR risks from food animals. Reduction in AMU/AMR indicates success of the industry action to contain AMR.

T2-11 Prevalence and Distribution of Antimicrobial-Resistant Bacteria in Brazilian Food Animal Production

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Introduction: Antimicrobial resistance is a worldwide concern and food animal production likely contributes to the increasing prevalence and distribution of resistant bacteria.

Purpose: We aimed to characterize the prevalence and distribution of antimicrobial-resistant bacteria in different Brazilian food production chains.

Methods: Fourteen food animal facilities (beef, pork, poultry, dairy and fish), from five Brazilian states (Minas Gerais, São Paulo, Mato Grosso do Sul, Paraná and Rio Grande do Sul) were visited to collect samples from animal feces, facility surfaces, animal water, animal carcasses, raw milk, end products, and worker feces (n=5,470 samples). Samples were streaked onto MacConkey agar and incubated at 37°C for 24 h. Isolates identified as *Escherichia coli* by biochemical test (n=7,630 isolates) were subjected to agar dilution assays to characterize their resistance to six antibiotics: amoxicillin (AMO, 8 µg/ml), ceftiofur (CEF, 8 µg/ml), ciprofloxacin (CIP, 1 µg/ml), chloramphenicol (CHL, 32 µg/ml), trimethoprim-sulfamethoxazole (SXT, 4/76 µg/ml) and tetracycline (TET, 16 µg/ml). Isolates with resistance to at least three antibiotics were considered as multidrug resistant (MDR).

Results: The prevalence of antimicrobial resistance was <15.0% for dairy and fish production. This was also the case for beef production except for a higher prevalence of AMO resistance for environmental and food samples (57.1% and 42.4%, respectively). Higher frequencies of resistance were observed for poultry isolates, especially from animal, environment and carcasses for SXT and TET (>40.0%). Isolates from pork presented the highest prevalence of antimicrobial resistance, especially for AMO, CHL, SXT and TET for all samples (16.0 to 83.0%). MDR was most common from pork (33.8 to 71.6%) and poultry (9.0 to 42.6%) isolates.

Significance: Pork and poultry production chains in Brazil may play an important role in the maintenance and dissemination of antimicrobial-resistant bacteria. Acknowledgments: CAPES, CNPq, FAPEMIG and FUNARBE.

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T2-12 Probiotic Bacteria to Reducing Antibiotic-Resistant Bacteria Transferred from Food Animals by Changes in Intestinal Flora

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Introduction: Abuse of antibiotics has increased antibiotic resistant pathogens in food, especially from food animals. Probiotics are increasingly being utilized as an alternative to antibiotics, because they can help improve the immunity by regulating the intestinal flora.

Purpose: The purpose of this study was to examine the effect of probiotic bacteria strains on increasing beneficial bacteria and diversity in gut microbiota and immune response in pigs.

Methods: A total of 60 weaning pigs at 4 weeks of age were fed either a basal diet[NC (negative control)] or a diet supplemented with probiotics [PC (positive control), commercial probiotics *Lactobacillus plantarum* LA; K, *Pediococcus acidilactici* K; WK1, *Pediococcus pentosaceus* SMFM2016-WK1; K-WK1, mix of *P. acidilactici* K and *P. pentosaceus* SMFM2016-WK1) for 4 weeks. Metagenome analysis was performed through Illumina 16S metagenomic sequencing to confirm intestinal microbial changes, and ELISA assay was performed to analyze cytokine (TNF-α, IL-12, IL-4, and IL-10) levels in serum.

Results: As a result of metagenome analysis, the microbial diversity and richness (Shannon index and Chao 1) of the PC and WK1 groups were higher than those of the other groups. The PC and WK1 groups had low dispersion, indicating that the similarity of the intestinal microflora between the experimental groups was greater than that of the other groups, according to the β-diversity analysis. In genus level, the ratios of butyric acid-producing bacteria *Roseburia* and *Eubacterium* was significantly higher (p<0.05) in the WK1 group than in the NC group. The level of pro-inflammatory (TNF- α, and IL-12) and anti-inflammatory (IL-4, and IL-10) cytokines of the PC and WK1 groups were similar to the NC group.

Significance: *P. pentosaceus* SMFM2016-WK1 can help increase beneficial intestinal flora and the diversity of intestinal flora, improving the immunity of pigs. Thus, it may decrease the use of antibiotics, reducing antibiotic-resistant bacteria from food animals.

T3-01 The Fate of Quinolizidine Alkaloids during the Processing of Lupins (*Lupinus* spp.) for Human Consumption

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Introduction: Lupin, a protein-rich grain legume, and products thereof are becoming increasingly important in our diets. However, variable and high concentrations of quinolizidine alkaloids (QAs) may hamper this evolution.

Purpose: This study assessed the fate of QAs when processing *Lupinus albus* seeds and lupin-based foods, to give a first indication of the food industry's ability to sufficiently reduce the QA concentration.

Methods: Typical unit processes, including toasting and dehulling, and the production of relevant food products, sterilized jarred lupins, cookies, chips and pasta, were simulated in duplicate on lab-scale. A quantitative determination of five QAs, i.e. sparteine, lupanine, lupinine, 13-hydroxylupanine and angustifoline, and qualitative screening of other relevant QAs, in the derived fractions and lupin-based foods, was performed with a validated UHPLC-MS/MS method and -HRMS method, respectively.

Results: Quinolizidine alkaloids are concentrated in the kernel of the seeds. As such, while a physical unit process such as dehulling will produce a protein-enriched fraction, the QA content will be enriched simultaneously. Furthermore, the depletion of QAs can be attributed to the leaching in cooking water. The production process of sterilized lupin beans depleted the QA content with over 60%, and the boiling of pasta confirmed that QA loss is caused entirely by the leaching of compounds into the cooking water, rather than a heat-induced degradation. Other heat treatments, including baking (dry) and frying (oil), had limited effect on the alkaloid concentration, indicating that QAs are heat-stable molecules.

Significance: These results are relevant in the ongoing search of the food industry for plant-based protein-rich foods and should be considered during the process of product development of innovative foods. These results are also of significant value for risk managers. As it will be important to set limits to protect consumers from a too high exposure to QAs, in case the consumption of these lupin-based foods increases.

T3-02 Evaluation of Gluten Protein Profiles in Hydrolyzed Food Products by a Multiplex-Competitive ELISA

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Introduction: Hydrolyzed foods and ingredients represent a small but important category that falls under the gluten-free regulation issued by FDA in 2020. Information is lacking on how hydrolysis affects different gluten proteins and the overall gluten protein/peptide profiles of hydrolyzed foods.

Purpose: Gluten protein/peptide profiles of several hydrolyzed food products were evaluated. The goal is to use the analysis results in the selection of appropriate calibrants for accurate quantitation of gluten in hydrolyzed food products.

Methods: The gluten protein/peptide profiles of 47 gluten-containing hydrolyzed foods (barley malt, sprouted wheat, hydrolyzed wheat proteins (HWP), sprouted rye, and sprouted barley) were evaluated using a multiplex-competitive ELISA. Cluster analysis was conducted to evaluate similarities or differences in gluten protein/peptide profiles among the hydrolyzed foods, and their similarities or differences with fermented foods analyzed previously by the ELISA. The suitability of a recently developed gluten-incurred yogurt calibrant for analyzing different fermented and hydrolyzed food products was also evaluated by cluster analysis. Clusters with AU (approximately unbiased) P>0.95 represented significant clusters and indicated products with similar gluten protein/peptide profiles.

Results: The apparent gluten concentration values of hydrolyzed foods ranged between 1-140,000 µg/mL depending on the type of foods analyzed, and the antibodies used. The gluten protein/peptide profiles, derived from the apparent gluten concentration values, mainly depended on the grain source (wheat, rye, or barley) of gluten. Some hydrolyzed foods presented profiles similar to fermented foods (e.g., barley malt and gluten reduced barley beers), whereas others presented unique profiles (e.g., HWP and sprouted wheat). Gluten incurred yogurts presented profiles similar to only wheat containing fermented and hydrolyzed food products tested (wheat beers, sourdough breads, sprouted wheat and HWP).

Significance: Grain specific calibrants are essential for accurate quantitation of gluten in certain hydrolyzed or fermented foods. While not suitable for the barley- or rye-containing foods tested, gluten-incurred yogurt calibrant showed promise for possible use in the quantitation of several wheat-containing fermented and hydrolyzed foods.

T3-03 Case Study on Novel Methodology for the Detection of Acrylamide in Food, Beverages and Water at the Point-of-Need Using the Micrylamid System

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Introduction: Acrylamide levels in food are of increasing concern due to carcinogenic and neurotoxic properties. Acrylamide forms in foods during the Maillard reaction at temperatures above 120$^{oC.}$ Current methods for detection of acrylamide are laborious and time-consuming. Point-of-need analysis brings the lab to the production facility, providing a quicker and easier method for the analysis of acrylamide.

Purpose: Case study on novel methodology for the detection of acrylamide in food, beverages and water at the point-of-need.

Methods: The MicrylaMiD system (Online-SPE-LC coupled with MS, Microsaic 4500 MiD®) was used for the analysis of acrylamide in samples such as coffee, crisps, and water. Acrylamide is concentrated using an activated carbon SPE cartridge and separated using a polar-C_{18} LC column. Minimal sample preparation is required, and low volumes needed (2 to 5 ml). Custom software capabilities create a fully integrated system that simplifies the interface for end users making it easy to use for non-experts, with result turnaround times of <10 minutes. Acrylamide standards are used for a calibration range of 0.5 to 800 ppb, in accordance with limits set in EU Regulation 2017/2158.

Results: This technique provides quick and easy quantification of acrylamide in food samples down to 0.5 ppb. The portability of the equipment enables it to be placed in a production facility or mobile van, reducing the pressure on a centralised testing laboratory.

Significance: The method enables acrylamide levels to be quantified at the point-of-need with a mobile system, producing quicker results that will ensure production is more easily monitored. Allowing for quicker mitigation of acrylamide production, this leads to a decrease in down-time in production with an instant impact on lowering acrylamide levels.

T3-04 Accumulation-Depuration Potential and Natural Occurrence of Microcystin-LR Toxin in Basil

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Introduction: Accumulation of hepatotoxic cyanobacterial toxins, like microcystin-LR (MC-LR), in edible crops through irrigation with contaminated water can result in human health risks.

Purpose: To assess the accumulation and depuration potential of MC-LR in basil under an optimized laboratory condition and to quantify its natural occurrence in basil plant samples collected from different markets in Belgium.

Methods: Basil plants in hydroculture were exposed to 5, 10 or 50 µg L^{-1} MC-LR for seven days. The depuration process was assessed by transferring plants to uncontaminated Hoagland solution for another seven days. Moreover, 50 basil products were collected from the Belgian markets. Basil leaves (lab and market) and roots (lab only) were analyzed using a validated UHPLC-MS/MS-based method to quantify MC-LR. ELISA and HRMS-techniques were applied to verify MC-LR presence in accumulation and depuration samples.

Results: Concentration dependent accumulation of MC-LR was observed in both basil leaves and roots, reaching for the highest treatment condition up to 87.90 µg kg^{-1} and 143.80 µg kg^{-1}, respectively. The basil roots accumulated more toxin compared to the leaves. Depuration was observed for all treatment conditions in both roots and leaves. At least six replicates were included and the whole experiment was repeated two times. These results were corroborated by both the ELISA and HRLCMS at the highest treatment condition. Moreover, MC-LR was detected below LOQ (1 µg kg^{-1}) in one market sample.

Significance: These results show the potential of basil to accumulate MC-LR from irrigation water, potentially resulting in human exposure to high levels of toxin. For the first time in Belgium, MC-LR was also detected in a vegetable from the market, showing human exposure through vegetables is already a reality.

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T3-05 Chemical Safety of Infant Formulas in Lebanon: A First-of-Its-Kind Study from the Arab World

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Introduction: Infant formula is a common source of food and nutrition for many infants and toddlers. However, the presence of contaminants may pose increased health risks to infants.

Purpose: A first of its kind investigation of the total concentrations of the metals and mycotoxins in infant formulae marketed in Lebanon was performed.

Methods: Toxic metals (Al, Ba, Cr, Pb, Cd and As) were analyzed with ICPMS, and mycotoxins (AFM1 and OTA) were analyzed using ELISA.

Results: As, Pb and Cd were observed in 59%, 8% and 10% of brands, respectively. Contamination levels were 31.0–1040 µg/kg, 38.0–476 µg/kg and 12.0–251 µg/kg, respectively. There was no significant difference (P<0.05) based on packaging, types, and classification. Samples which contained detectable levels of Pb, Cd and As had higher concentrations of these metals compared to breast milk, and infants have a higher average weekly intake of these metals through infant formula. On the other hand, all brands contained detectable concentrations of Al and Ba, Cr was detected in 95% of brands. Ranges were 0.080-7.93 (Al), 0.038-5.35 (Ba), and 0.041-0.348 (Cr) µg/g. A significant difference in the mean concentrations of Al, Ba, and Cr for the two production dates of a single brand was observed in 92, 59, and 83% of samples, respectively. For mycotoxins, mean (±SD) of AFM1 and OTA concentrations were 20.1±1.3 ng/kg and 0.37±0.10 µg/kg, respectively. 13 (31%) and 14 (33%) brands had an average level above EC limit for AFM1 and OTA, respectively. There was a significant (P<0.05) difference between production dates for AFM1, but not for OTA. No significant (P<0.05) difference was found between fall/winter and spring/summer for both mycotoxins.

Significance: Our study provides the first publicly available information on metal contamination in infant formulas in the region and reveals the need for frequent monitoring and surveillance of these products.

T3-06 Acrylamide Reduction via Asparaginases in Cookies Can be Improved Depending on Baking Conditions and Incubation Temperature

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❖ ***Developing Scientist Entrant***

Introduction: In heated food, the Maillard reaction between asparagine and a carbonyl source can result in formation of acrylamide (AA), which is a toxic and probably human carcinogenic molecule. Due to high levels of free asparagine (precursor) in cereals, AA remains a food safety concern in the industry. Although there are already a number of AA mitigation measures in use, most of them are implemented through a trial-and-error process due to the lack of systematic investigations.

Purpose: Therefore, the aim of this study is to investigate the effect of asparaginases in different concentrations, incubation times and temperatures for reduction of AA in cookies, 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. We analyzed a total of 288 samples.

Results: Here we show that two commercially available asparaginases significantly reduced acrylamide (up to 85%), while the color was not affected by their addition, nor the texture of the cookies. Incubating cookie dough at 10 and 30 minutes did not affect the AA formation as much as the incubation temperature of 90°C did, compared to 60°C. Increasing the baking temperature from 180°C to 240°C showed only slightly a higher AA formation, due to the concomitant reduction in baking time. However, using a heat-stable asparaginase at temperatures above 200°C was more efficient regardless of the concentration, 100 or 200 ppm, respectively. The outcomes support the advantages of using asparaginases, because the recipe and process conditions remain the same and the product has the same textural properties and no color change after baking.

Significance: This research highlights its significance in maintaining AA levels under benchmark levels with low effort and therefore providing safe food for consumers.

T3-07 A Flexible Bacterial Cellulose-Based SERS Substrate for Rapid Determination of Thiram on Apple Surface

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❖ ***Developing Scientist Entrant***

Introduction: Traditional analytical methods for determining pesticide residues face the challenge of complex and lengthy sample preparation.

Purpose: The objective of this study is to develop a rapid and simple method for the determination of thiram on apple surface using a flexible bacterial cellulose-based SERS substrate.

Methods: This BC-based SERS substrate was fabricated by immersing BC film into AuNPs solutions. In the fabricated substrate, BC film with a fine 3D network structure acted as a platform to absorb gold nanoparticles uniformly, that are responsible for the enhancement of Raman scattering signals. Based on the developed substrate, a bench-top Raman spectrometric system was successfully applied to detect trace thiram on apple surface via two different collection methods (i.e. "press-and-peel" and "wiping"). In addition, a portable Raman spectroscopic device was used to detect thiram on apple surface to achieve on-site applications. The overall test of thiram on apple surface can be completed within 8 min including sample preparation (3 to 5 min) and SERS measurement (1 to 3 min).

Results: Enhancement factor of this SERS substrate for sensing thiram molecules was calculated to be 2.8×10^5. The limit of detection of thiram residues determined by a portable Raman spectroscopic device and a benchtop Raman spectrometer were 0.98 ppm and <0.5 ppm, respectively, meeting maximum residue level of thiram on apple according to the regulation of Europe and North America. SERS peak intensity at 1368 cm^{-1} was proportional to thiram concentration (1 to 50 ppm) with a coefficient up to 0.99. The analytical performance of the flexible BC-based SERS substrate was verified in terms of sensitivity, reproducibility and stability.

Significance: The developed method can be used to determine pesticide residues on food surface rapidly and non-destructively (<8 min), ensuring food safety.

T3-08 Gaseous Chlorine Dioxide Used for Improving the Safety and Shelf Life of Grape Tomatoes

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Introduction: Grape tomatoes have been associated with outbreaks due to the contamination of foodborne pathogens such as *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. Chlorine dioxide (ClO_2) is known to be an excellent disinfectant and its bactericidal properties are well documented. However, ClO_2 is a volatile gas that must be produced on-site and required sophisticated chemical generation equipment.

Purpose: This study was to develop packaging films with sodium chlorite (NC) and polylactic acid (PLA) polymer, which released gaseous ClO_2 to inactivate these pathogens and spoilage microflora on tomatoes.

Methods: NC was integrated into PLA films using mixing-casting method. The antimicrobial film was placed inside PET clam shell containers with grape

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tomatoes. Stem scars of tomatoes were inoculated with *E. coli* K12, *Listeria innocua*, *E. coli* O157: H7, *Listeria monocytogenes* and *Salmonella* Enteritidis. The inactivation of these bacteria on tomato stem scars by the films were investigated. Non-inoculated tomatoes were also used for the shelf life study.

Results: The film treatments reduced the populations of *E. coli* K12 by 4.0 log CFU/g, and *L. innocua* by 2.2 log CFU/g. Similarly, the film treatments reduced the populations of *E. coli* O157:H7 by 4.3 log CFU/g, *L. monocytogenes* by 2.4 log CFU/g, and *Salmonella* by 4.0 log CFU/g. After stored for 2 weeks at 4°C for 2 weeks, film-treated tomatoes had native bacterial (TPC) populations of 1.8 log CFU/g and native yeast and mold (Y&M) populations of 2.6 log CFU/g, respectively, while the control samples had 4.8 log CFU/g of TPC and 4.5 log CFU/g of Y&M. Control samples also showed moldy surface after 2 weeks. In contrast, treated tomatoes still had fresh-like appearance after 2 months.

Significance: This study demonstrates that the use of NC-PLA antimicrobial films is a simple and effective way to enhance the safety and extend the shelf life of grape tomatoes.

T3-09 Development of Continuous- and Self-Sanitizing Surface Coatings Based on Visible Light to Prevent Cross-Contamination

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Introduction: Cross-contamination of food with pathogens, including bacteria and viruses, is a major cause of food-borne disease outbreaks across multiple segments of the food industry. The risk of cross-contamination is significant in diverse environments including harvesting, post-harvest processing and food service industries.

Purpose: This work was focused on development of antimicrobial “slippery” coatings using photoactive food grade compositions to reduce biofouling, improve microbial inactivation and provide continuous- and self- sanitation surfaces.

Methods: Nanofibrous membranes were functionalized with fluorosilane (approved for food contact surfaces) for reducing the surface energy and curcumin for the daylight-induced photoactive antimicrobial function. A nanofibers suspension was used as a spray coating on the stainless-steel surface, followed by silicone oil infusion as a lubrication agent to create the antimicrobial slippery surfaces (SLIPS). The antimicrobial activity of the coated surfaces was investigated against *Escherichia coli* O157:H7 and *Listeria innocua*. The anti-cross-contamination property of the designed surfaces were examined by conducting spinach leaf-surface-leaf cross-contamination test.

Results: The designed surface showed superhydrophobic property with a contact angle of 166°. Without the antimicrobial function, SLIPS surface significantly reduced the cross-contamination risk, reducing more than three and four logs of bacterial transfer from the contaminated spinach leaf to the SLIPS surface and the non-infected spinach leaf respectively. With curcumin modification, the designed surface exhibited excellent self-sanitation property by reduction of 7 log (99.99999%) of both tested microbes after daylight irradiation time of 10 min. After continuous daylight exposure for seven days, the prepared antimicrobial SLIPS exhibited good photostability and retained its light-induced self-sanitation power. Furthermore, the designed surface showed self-sanitation durability by reduction of 7 log of *L. innocua* for five consecutive cycles.

Significance: This work illustrates the development of continuous and self-sanitation of surfaces in food processing and food service environments that can reduce the risk of microbial contamination and improve the food safety and quality.

T3-10 Global Distribution of Genes Conferring Increased Tolerance to Food Industry Disinfectants in *Listeria monocytogenes*

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Introduction: Genes associated with increased *Listeria monocytogenes* tolerance to one of the most common food industry disinfectants, quaternary ammonium compounds (QACs), have been described but their global distribution in *L. monocytogenes* has not been examined to date.

Purpose: To characterize the dissemination of QACs tolerance genes in public *L. monocytogenes* raw sequencing data.

Methods: Sequencing runs of 39,196 *L. monocytogenes* isolates deposited in the European Nucleotide Archive until April 2021 were screened for presence of *bcrABC*, *qacH*, *emrC* and *emrE* genes using COBS and KMA. COBS was used with default settings, while KMA screening was carried out with minimum template identity of 90%. MLST typing was performed by stringmlst against the *L. monocytogenes* MLST database. The heterogeneity in proportion of clonal complexes (CCs), geographical locations and isolation sources that were positive within and between genes was estimated using a random-effects model implemented in the R package meta.

Results: QACs tolerance genes were detected in 10,953 (28%) of the *L. monocytogenes* isolates. *bcrABC* was the most abundant gene, present in 72% of the QACs positive isolates, followed by *qacH* (19%), *emrC* (7%) and *emrE* (2%). The prevalence of all genes, except *emrC*, was significantly higher ($P<0.05$) in food and production environment isolates, while *emrC* was detected mostly in clinical samples. We found significant differences ($P<0.05$) in the dissemination of the QACs genes among countries/continents and CCs. While *bcrABC* was highly prevalent in the United States, *qacH* and *emrC* were associated with Europe and *emrE* had highest occurrence in Australia/Oceania. *emrE*, *emrC* and *qacH* were strongly associated ($P<0.05$) with CC8, CC6 and CC121, respectively, while *bcrABC* was distributed among several CCs with highest occurrence in CC5, followed by CC321, CC155, CC9 and CC7.

Significance: This study gives a comprehensive overview of the dissemination of QACs genes globally and exemplifies the importance of sharing sequencing data.

T3-11 Antivirulence Effect of Cannabidiol Against *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a serious food-borne pathogen causing meningitis and death in humans, especially in susceptible individuals. *L. monocytogenes* infection begins with its colonization in the intestinal tract followed by systemic spread. The major virulence factors associated with *L. monocytogenes* infection include bacterial motility, hemolysin and lecithinase production, and invasion of host tissue, including intestinal cells and the central nervous system. Reducing the activity of these virulence factors would help to control *L. monocytogenes* pathogenesis and improve disease outcome.

Purpose: This study investigated the anti-virulence effect of Cannabidiol (CBD), a major non-psychoactive constituent of *Cannabis sativa* on *L. monocytogenes*.

Methods: Sub-inhibitory concentration (SIC, concentration not inhibiting bacterial growth, 11.92 µM) and minimum inhibitory concentration (MIC) (2289-2607 µM) of CBD against three *L. monocytogenes* isolates, namely Scott A, ATCC 19115, and LM1 were determined. The three isolates were grown in TSB with or without SIC, 2xSIC, 3xSIC, 4xSIC, 5xSIC, 6xSIC, 1/16 MIC, 1/8 MIC, 1/4 MIC, 1/2 MIC, and MIC of CBD to assess bacterial motility, hemolysin production and lecithinase activity. The effect of CBD on LM adhesion and invasion of human colon adenocarcinoma cells (Caco-2) and human brain microvascular cells (HBMEC) was also determined. Additionally, the effect of CBD on major LM virulence genes was analyzed using RT-qPCR. All experiments were repeated thrice with duplicates and data were analyzed using one-way ANOVA.

Results: Cannabidiol from 5x SIC to MIC significantly reduced all LM virulence factors tested ($P< 0.05$). At MIC, CBD decreased motility and hemolysin by 100%, lecithinase production by 50-60%, and adhesion and invasion of intestinal epithelial and brain microvascular cells by 50-70% ($P<0.05$). RT-qPCR data revealed that SIC of CBD downregulated the transcription of LM virulence regulator (*prfA*) and genes, *hly*, *plcA*, *plcB*, *iap*, *motA* and *motB* at least by two-fold

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(P<0.05).

Significance: Cannabidiol could potentially be used as a therapeutic agent against *Listeria monocytogenes*, and follow-up *in vivo* studies are required for validating these results.

T3-12 Is Your Food Really as Cool as You Think It is? ...Putting Inertia Temperature Sensor Technology to the Test

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Introduction: It is a well understood fact that food refrigeration systems are designed to keep food cooler than the prevailing temperature at the location where the refrigeration system is situated. Also well understood is that food is kept cold for several reasons including food safety, preservation of freshness, compliance, and customer satisfaction. Historically the refrigerated food holding environment has been monitored by placing air temperature sensors within the appliance.

Purpose: What is not well understood, is that the temperature of the air in the refrigerated enclosure may be a highly inaccurate measure of the actual food items temperature inside the refrigerated enclosure. In reality the air temperatures within the appliance can fluctuate dramatically due to door openings, defrost cycles, power outages, overstocking, etc. The main aim is to keep stored 'products' at a predetermined target temperature, without being overly reactive to changes in transient air temperatures.

Methods: A revolutionary new approach to food temperature monitoring is being introduced in the form of wireless thermal inertia sensors. The In-dentiCool line of sensors incorporates thermally engineered fluids that closely replicate the thermal properties of the foods being monitored. Critical food safety decisions should always be made based on the temperature of the food and not the air temperature in the appliance.

Results: Extensive laboratory and field data will be presented that shows how this new temperature monitoring approach has resulted in the reduction of shrink, increased regulatory compliance, power savings, and overall smoother facility operations.

Significance: The implementation of wireless food simulant enabled temperature sensors is a significant step forward in the science of cold holding. The continued practice of relying on air temperature sensors in the cold holding asset can lead to appliance inefficiencies, improper food temperature data, and menacing food holding conditions.

T4-01 Any Hand Hygiene Intervention is Better Than No Hand Hygiene Intervention – A Systematic Study to Evaluate the Use of Alcohol-Based Hand Sanitizers in a Simulated Retail Food Preparation Setting

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Introduction: Per the FDA Food Code (FC), alcohol-based hand sanitizers (ABHS) cannot be used in lieu of handwashing (HW) during food preparation. A recent study investigated the impact of hand hygiene interventions (HHI) on microbial cross-contamination to surfaces and hands during food preparation (see complementary posters), finding no difference in the performance of ABHS vs HW.

Purpose: Identify associations between worker behaviors, HHI compliance, and microbiological contamination of surfaces and hands.

Methods: Food handlers (n=85) were assigned to one of five groups: control (no HHI); FC-directed HHI (HW or ABHS); and natural HHI (no direction; with and without access to ABHS). Workers prepared meals using raw meat containing *Escherichia coli* DH5-α (NCSU IRB #21056). All meal preparations were recorded and coded. Afterwards, surface swabs (n=850) and hand rinsates (n=85) were collected and screened for *E. coli* contamination. Associations were determined using one-way ANOVA and Chi-square tests.

Results: Compared to treatment groups, the absence of HHI (control group) led to significantly higher *E. coli* contamination on surfaces (P<0.001) and hands (P<0.05). Behavior groups, receiving no FC-HHI direction, had statistically lower HHI attempts and successful compliance according to FC-guidelines compared to FC-HHI directed groups (P<0.001), however, surface and hand contamination did not significantly differ between HHI treatments (P>0.05). *E. coli* concentration on contaminated hands was similar when workers had access to ABHS (2.6±0.7 log CFU/rinsate) compared to the FC-HHI directed HW group (2.6±1.0 log CFU/rinsate; P=0.958).

Significance: Our research showed that any HHI, including ABHS, reduced microbial contamination on kitchen surfaces and hands of food workers, regardless of the number of successful HHI attempts performed according to FC-guidelines. These results suggest inclusion of ABHS may be a viable option in food service in certain situations, but additional studies are necessary.

T4-02 Microbial Disinfection of Food-Contact Surfaces Using a Germicidal Short-Wave Ultraviolet Light (279 nm) Emitting Diode System

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Introduction: The need for sustainable UV-C technologies for the food-contact surface to minimize the risk of cross-contamination is investigated. Novel decontamination approaches to inactivate these resilient bacteria are researched to enhance food safety.

Purpose: The objective of this research was to determine the UV-C dose-response curve of *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 700720), and *Listeria Monocytogenes* (ATCC 19115) on food-contact surfaces using an ultraviolet light emitting diode (LED) operating at 279 nm wavelength.

Methods: 50µl cell suspension was spread uniformly over a 1 cm circular stainless-steel coupon and treated using a near collimation LED apparatus with UV-C doses of 0, 2, 4, 6 & 8 mJ/cm (n=3). After UV-C exposure, enumeration of microorganisms was performed by serial dilution plate count method (detection limit 2 log CFU/ml). All experiments were conducted in triplicate. For UV inactivation kinetic modeling, the GinaFit tool was used and reported goodness of fit (R^2) and RMSE.

Results: Both *Escherichia coli* and *Salmonella typhimurium* inactivation followed log-linear kinetics with R^2 of 0.99 and RMSE between 0.10 and 0.12. In contrast, UV inactivation of *Listeria monocytogenes* showed non-linear kinetics (Weibull) with R^2 of 0.99 and 0.15 RMSE. The dose required for three-log reduction of the *Escherichia coli*, *Salmonella typhimurium,* and *Listeria monocytogenes* was 8.09±0.3, 5.71±0.2 and 5.02±0.2 mJ/cm respectively. *Listeria monocytogenes* exhibited the highest UV sensitivity followed by Salmonella *typhimurium* and *Escherichia coli* had the lowest UV-C sensitivity. Overall, the data show UV-C light at 279 nm efficient against target micro-organisms.

Significance: This kinetic data is useful for calculating the desired UV-C doses for bacterial log reductions on food-contact surfaces.

T4-03 Efficacy of Acidified Water-in-Oil Emulsions Against Desiccated *Salmonella* as a Function of Osmotic Pressure, Acid Carbon Chain-Length, and Cellular Membrane Fluidity

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Introduction: Sanitizing low-moisture food (LMF) processing equipment is challenging due to the increased heat resistance of *Salmonella* spp. in low-water activity (a_w) environments. Our lab has developed antimicrobial oils and water-in-oil (W/O) emulsions with acetic acid, which were effective against desiccated *Salmonella*.

Purpose: To evaluate the efficacy of W/O emulsions with different carbon chain-length (C_n) organic acids against desiccated *Salmonella*.
Methods: Salmonella Enteritidis was inoculated onto stainless-steel coupons and desiccated at 75% relative humidity (RH). Treatments were 200 mM C_{1-12} organic acids prepared in W/O emulsions (3% PGPR, 1% water) and W/O emulsions with glycerol (3% PGPR, 1% water, 3% glycerol), with a contact time of 30 min at 22 °C or 45 °C. Microbial log reduction (MLR) was determined with plate counts and Most Probable Number. Fluorescence lifetime imaging microscopy (FLIM) was used with BODIPY-FL-C_{12} to evaluate cellular membrane viscosity. Differences were significant at *P*<0.05. Two-way ANOVA with post-hoc Tukey was used to denote levels of statistical significance.
Results: At 22 °C and 45 °C, desiccated *Salmonella* was found highly susceptible (>6.5 MLR) to the W/O emulsions formulated with C_{1-3} acids. Those formulated with C_{4-12} acids had little to no efficacy at 22 °C, but were effective (> 6.5 MLR) at 45 °C. Experiments with FLIM showed that *Salmonella* stained with BODIPY had a shorter fluorescence lifetime at 45 °C, compared to 22 °C. Thus, we hypothesized that heating of *Salmonella* decreases membrane viscosity, allowing the longer chain-length acids to permeate the cells. All acids in W/O emulsions were attenuated with glycerol at 45°C, suggesting that the antimicrobial mechanisms may be due to differential osmotic pressure and acid membrane permeability.
Significance: Alcohol-based sanitization used commercially for LMF processing requires system cooldown due to flammability. Antimicrobial oils and W/O emulsions can be used at elevated temperatures and thus prevent production downtime.

T4-04 Using Plasma-Activated Water (PAW) for Disinfection of Common Material Surfaces in Poultry Houses

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* spp. is considered as the major source of food illness in poultry industry. Current procedures used for disinfection are expensive and not always effective. The potential to use only air, water, and small amount of electricity to generate an on-site disinfectant at lower costs is preferred.
Purpose: The purpose is to evaluate this specific PAW for disinfection of stainless-steel, polyvinyl chloride (PVC) and wood surfaces found in poultry houses.
Methods: PAW was prepared by treatment of 24 ml water samples with high voltage atmospheric cold plasma (HVACP) in 80 % humid air (working gas) under an applied voltage of 90kV for 30 minutes with an average power of 168 W. Stainless-steel, PVC (5 by 1.25 cm) and wood (6.25 by 1 cm) coupons representing materials which usually occur in poultry houses were inoculated with 10 μl (stainless-steel and PVC) and 100 μl (wood) of *Salmonella* cocktail (*S*. Enteritidis, *S*. Typhimurium, *S*. Montevideo, and *S*. Newport) with a final microbial load of 9 log CFU/ml and treated with 2 ml of PAW for 30 s. Then surface was swabbed with cotton swabs and *Salmonella* counts were determined on Tryptic Soy Agar. The trials were repeated independently five times and a one-way ANOVA was performed. Stainless-steel coupons (under the same conditions) were also treated with PAW stored at 4 °C for 7 and 14 days. Inoculated non-treated control samples were subjected to the same treatment conditions with deionized water instead of PAW.
Results: The significant reductions (*P*<0.01) 2.96±0.47, 3.16±0.48 and 1.05±0.63 log CFU/ml were achieved after 30 s of treatment for stainless steel, PVC and wood, respectively. PAW stored at 4°C for 7 and 14 days when applied to stainless steel reduced *Salmonella* by 2.97±0.30 and 2.99±0.33 log CFU/ml, respectively.
Significance: PAW generated from HVACP treatment can produce a disinfectant suitable for decontaminating poultry houses.

T4-05 Investigating Current Low-Moisture Food Processing Environment Sanitation Practices Against Dry Surface Biofilms of Listeria monocytogenes, Salmonella enterica Serovar Typhimurium, and Pseudomonas aeruginosa

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Introduction: Current low-moisture food (LMF) sanitation practices may not be sufficient to identify and deactivate dry surface biofilms (DSB) and requires verification against pathogens persisting as DSB.
Purpose: To test bactericidal efficacy of EPA registered isopropyl alcohol sanitizer with LMF claim and microfiber swab ability to remove/displace bacterial populations within DSB.
Methods: Mono-and mix-culture wet surface biofilms (WSB) of *Salmonella enterica* ser. Typhimurium ATCC 700720, *Listeria monocytogenes* FSL R8-5318, and *Pseudomonas aeruginosa* ATCC 15442 were developed following standard EPA protocol on borosilicate glass coupons using CDC® biofilm reactor. WSB were dried for 24, 48, and 72 h at 21°C to form DSB and treated with microfiber swabs or 10% isopropyl alcohol sanitizer. Serial dilutions were plated on selective agar for each species. Mean log CFU/cm^2 were calculated and compared to control (PBS) using LSM of PROC GLM to fit linear models (a=0.05). Replications were done in triplicate.
Results: There were no significant differences in the mean log densities/coupon between control and coupons cleaned with microfiber swabs to displace mono-and mix-culture DSBs (*P*>0.05). Although isopropyl sanitizer reduced WSB *S. enterica* populations to approx. four logs (*P*<0.05), the bactericidal efficacy ranged between 1 to 3 logs when co-cultured with *P. aeruginosa* and as dehydration time increased. *P. aeruginosa* populations alone and when co-cultured with *L. monocytogenes* recovered post-isopropyl alcohol sanitizer treatment were not significantly different from control coupons (*P*>0.05) suggesting it was more difficult to deactivate *P. aeruginosa* DSB and could exhibit protection to underlying *L. monocytogenes* populations.
Significance: Considering emergence of LMFs as potential sources of foodborne outbreaks and recalls, our data will provide insights to the gaps in the current sanitation practices within LMF processing facilities that may harbor pathogens and propose models that support mitigation strategies.

T4-06 Repeated Disinfection with Industrial Biocides Alters the Composition and Biocide Tolerance in Mock Drain Biofilms

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Introduction: Residual biofilms challenge effective sanitation in the food industry.
Purpose: This study aimed to investigate if repeated disinfection with industrial biocides altered the microbial community composition and biocide tolerance in mock drain biofilms.
Methods: A mock drain biofilm composed of 31 representative bacterial species isolated from metagenomically characterized industrial floor drains, was created (3 days, 15°C) on stainless steel plates in tryptic soy broth (TSB). Biofilms were subsequently exposed for five min to water (control) or low, high or 2x high industrial concentrations of benzalkonium chloride (BC, 250, 1000, 2000 ppm), peracetic acid (PAA, 500, 2500, 5000 ppm) or sodium hypochlorite (SH, 1000, 6000, 12000 ppm). Survivors were enumerated using tryptic soy agar before and after disinfection, following biofilm regrowth (3 days, 15°C, TSB) and repeated disinfection treatments. Changes in biofilm microbiome were determined using MALDI-TOF to identify survivors (n=160) after treatments with water or high biocide concentrations. Survival of *Listeria monocytogenes* were evaluated based on selective enrichment.
Results: The initial mock drain biofilm (6.9 ± 0.3 log CFU/cm^2) exhibited small reductions of 1.8 log CFU/cm^2 after exposure to low concentrations of PAA and SH and all concentrations of BC. Higher concentrations of PAA and SH reduced the biofilm by 3.4 to 4.5 log CFU/cm^2. Disinfected biofilms regrew to 7.6 ± 0.5 log CFU/cm^2. The nine treatments led to three cases of increased tolerance and one case of increased sensitivity of the biofilm to the second biocide

exposure. Eradication of *L. monocytogenes* required two exposures to 5000 ppm PAA. *Pseudomonas* spp. became highly abundant (25 to 70%) after biocide exposures. Interestingly, *Serratia* spp. (40%), *Acinetobacter* spp. (53%) and *Psychrobacter* spp. (39%) dominated biofilms after the second exposure to BC, PAA and SH, respectively.

Significance: In conclusion, high concentrations of SH and PAA effectively reduce biofilms populations. However, repeated exposure altered the surviving microbiome and fostered increased biocide tolerance.

T4-07 *Listeria monocytogenes* Colonizes Biofilms in Floor Drains and Its Prevalence Correlates to Aerobic Plate Counts and Biomass

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Introduction: The ability of *Listeria monocytogenes* to persist in food processing environments is enabled by inclusion into biofilms yet its role in microbial communities is not well understood.

Purpose: To characterize biofilm development in retail deli floor drains and *L. monocytogenes* inclusion in the microbial community.

Methods: Sterile stainless steel coupons were attached to the inner wall of deli floor drains in eight locations across three midwestern states. Beginning at six weeks post-installation, two coupons per drain were removed for analysis and the drain cover was swabbed. Coupons were rinsed and one was placed in an oven at 60°C and weighed repeatedly until stable. The biofilm was removed and the coupons weighed. The other coupons' biofilm was resuspended in PBS for APC. The suspension and drain swab were tested for *L. monocytogenes*. Samples were collected and analyzed bi-weekly for 16 weeks.

Results: APC counts differed over time (χ^2; *P*=0.0007) with weeks 14 and 16 higher than the prior four sampling events. Dry weight was highly variable and only weeks 14 and 6 differed (F; *P*=0.0040). *L. monocytogenes* was present in 27.1% (13/48) of the biofilm samples and 22.9% (11/48) of the drain cover swabs. *Listeria*-positive biofilms APC counts rose over time in linear trend (Z; *P*=0.0224) but this was not observed of the swabs (Z; *P*=0.9984). There wasn't a correlation between a samples' swab and biofilm results (K; *P*=0.9872). *Listeria*-positive biofilm samples had significantly higher plate counts (χ^2; *P*=0.0286) and dry weights (χ^2; *P*=0.0286) than the negatives.

Significance: L. *monocytogenes* inclusion into biofilms is related to measures of biofilm maturity and composition.

T4-08 Resistance of Salmonella Tennessee and Salmonella Typhimurium Strain LT2 Biofilms to Industrial Antimicrobials Highlights the Importance of Preventive Measures

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Introduction: Capability of *Salmonella* to form biofilms on various biotic and abiotic surfaces is an important underlying reason for prevalence of the pathogen. Biofilms are estimated to be causative agents of around 80% of bacterial infections in humans.

Purpose: The current study compared sensitivity of an epidemiologically significant serovar (*Salmonella enterica* subspecies *enterica* serovar Tennessee) to two industrially important antimicrobials at various stages of biofilm formation. Suitability of an avirulent strain (*Salmonella enterica* subspecies *enterica* serovar Typhimurium, strain designation LT2) as a surrogate microorganism was additionally studied.

Methods: Biofilm trials were randomized block designs with two biologically independent repetitions (each containing three replicates) as the blocking factor. The log-transformed bacterial counts were statistically analyzed (at 5% type I error) using Tukey-adjusted ANOVA. Biofilm trials consisted of the application of sterilized water (treated control), sodium hypochlorite, and quaternary ammonium compounds with the two latter tested at the highest concentrations recommended by manufacturers. Using the glass beads method, the sensitivity of planktonic cells and various stages of biofilms were studied.

Results: Our results illustrate that using industrially important antimicrobial treatments at the highest concentrations suggested by the manufacturers is only efficacious ($P<0.05$) against planktonic and one-day mature biofilms of the pathogen while exhibiting a lack of efficacy ($P\geq0.05$) for complete removal of bacterial biofilms formed for more than 2 days. Similarities ($P<0.05$) between the pathogenic and non-pathogenic strains further indicate that *Salmonella* LT2 could be used as a surrogate for pathogenic *Salmonella* in public health microbiology validation studies.

Significance: These findings show the importance of including both planktonic and sessile cells of the pathogen in microbiology hurdle validation studies, especially for treatments of niche and hard-to-reach surfaces. Resistance of biofilms of this epidemiologically important strain to industrial treatments highlights the importance of preventive measures against this opportunistic and prevalent pathogen of public health concern.

T4-09 The Risk Assessment of the Sanitation Practices of Modified Washing Machines in the Processing of Leafy Greens

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Introduction: About 46% of foodborne illness incidents are attributed to raw produce-like leafy greens. Small-sized growers retrofit washing machines to dry triple-washed leaves, effectively turning them into large salad spinners. Previous work demonstrates that this practice has the potential to cause cross-contamination of *Listeria*. However, the potential efficacy of different cleaning agents and sanitizers to mitigate this risk has not been explored.

Purpose: This project aims to investigate various risk reduction mitigation strategies using traditional on-farm applications of cleaning agents and investigates two commonly used food contact surface sanitizers.

Methods: Listeria innocua at 10^2-10^9CFU/ml was inoculated and dried onto spinach, followed by drying in a retrofitted washing machine modified for postharvest drying of leafy greens. The application of detergent and water with mechanical action like scrubbing to evaluate the cleaning and sanitizer (chlorine (200 ppm) and peroxy acetic acid (100 ppm) based sanitizers) efficiency. Three contact surfaces per layer of the machine were sampled using sterile microbial swabs and ATP swabs to enumerate the relative levels of the bacterial transfer and ATP recovered.

Results: There was a 90%±5% microbial recovery from the different contact surfaces of the machine. Overall, the highest level of recovery was consistently measured in the bottom water-collecting contact point, suggesting the highest risk of bacterial deposition may occur in this region. The mitigation strategies were proven to be successful as the microbial recovery was below the LOD post-cleaning and residual microorganisms were completely removed post-application of both chlorine and PAA sanitizers. In addition, the ATP values were reduced to approximately 150 RLU post-cleaning.

Significance: This information supports the need for producers to establish a cleaning and sanitizing SOP for drying units to reduce microbial contamination in post-harvest surfaces such as using modified washing machines.

T4-10 Automated Floor Cleaning Reduces *E. coli* Spread Compared to Mechanical Deck Brushing

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Introduction: The use of automated machines on non-food contact surfaces (e.g., floors) in food facilities and healthcare systems has been avoided due to concern of spreading pathogens (e.g., aerosols); there is limited data informing this concern.

Purpose: To investigate differences in mechanical and automated cleaning/sanitizing methods ability to remove and spread ampicillin-resistant *Escherichia coli* from floor surfaces.

Methods: Taski Swingo µicro auto scrubber (Diversey Inc, USA) and a deck brush were compared using a quaternary ammonium compound sanitizer on a two m^2 vinyl floor. Fifteen locations, three in the initial inoculation zone and three every 0.5 m^2, were inoculated with $3x10^7$ CFU *E. coli* and dried for 30 min before sanitation. Settle plates and a Spin air basic air sampler (Neutec Group Inc, USA) were used to enumerate *E. coli* in aerosols generated during sanitation. Samples were plated or collected on LB agar with 100µg/mL ampicillin and incubated for 24 h at 37°C. Testing was done in triplicate and statistical analysis was completed in SAS.

Results: The auto scrubber and sanitizer combination exhibited a significantly higher bactericidal efficacy on the floor compared to the deck brush (*P*=0.003). There was a significant difference (*P*=0.0072) among equipment parts when tested for cross-contamination. The machine squeegee had the least contamination (0.55 log CFU/ml), while the deck brushes had an average of 2.72 log_{10} CFU/mL residual contamination. Limited *E. coli* was detected by the air sampler and settle plates when using the auto scrubber. During deck brushing, more *E. coli* was detected on settle plates compared to the air sampler (*P*<0.0001).

Significance: Automated scrubbing minimized bacterial spread in the environment and effectively eliminated *E. coli* from the floor. Furthermore, it performed significantly better than deck brushing and may serve as a possible opportunity to reduce the environmental spread of bacteria when cleaning.

T4-11 Effective Strategies to Sanitize Harvesting Bins and Picking Bags Concerning *Listeria monocytogenes* and *Salmonella*

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Introduction: Food contact surfaces used during harvest such as picking bags and storage bins can cross-contaminate produce if not properly cleaned and sanitized. Thus, there is a need for the development of cleaning and sanitary controls which effectively inactivate target foodborne pathogens and can be applied in the field and packinghouse.

Purpose: Evaluate the effectiveness of commercially available sanitizers in reducing *Listeria monocytogenes* and *Salmonella* sp. on experimentally inoculated coupons representative of food contact surfaces commonly used in harvesting bags and bins in the apple industry.

Methods: Wood, plastic (HDPE), and nylon coupons were spot inoculated with a cocktail (8.5 log CFU/ml) of *L. monocytogenes* (390-6 (serotype 1/2a),390-2 (serotype 1/2b), 573-035 (serotype 4b)) or *Salmonella* (Enteritidis ATCC BAA-1045, Agona LJH 517, Newport ATCC 6962) and allowed to dry prior to treatment. Three sanitizer treatments [peroxyacetic acid (PAA; 500 ppm), free chlorine (500 ppm), and silver dihydrogen citrate (SDC; 0.0003% silver ions 4.846% citric acid)] and steam were evaluated. Coupons were exposed to steam for 30 s and 1 min while contact times for sanitizers were 1 and 2 min. Microbial populations were enumerated and compared to no treatment controls to determine significant differences (*P*<0.05) in a completely randomized design (n=9).

Results: Application of SDC, PAA, and chlorine at contact times of 1 and 2 minutes on plastic and nylon coupons resulted in a >4 log reduction in *L. monocytogenes* compared to water-only controls without any significant differences amongst sanitizer chemistries (*P*>0.05). *Salmonella* on plastic material for treatments SDC, PAA at 1 and 2 minutes and chlorine at 2 minutes showed a >5 log reduction without any differences amongst approaches (*P*>0.05). Steam was the least effective treatment evaluated (<4 log reduction). Sanitizer applications on wood material showed a log reduction of a < 3 (*P*<0.05).

Significance: This study helps provide scientific evidence for efficacy of different sanitizing practices for harvest bins and picking bags in the tree fruit industry.

T4-12 Evaluating the Cleaning and Sanitation Practices of Fresh Produce Farms and Packinghouses in the Pacific Northwest

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❖ ***Developing Scientist Entrant***

Introduction: Inadequate cleaning and/or sanitation (C&S) of food contact surfaces (FCS) has been frequently reported during Produce Safety Rule inspections; however, limited data are available to support or refute effectiveness of C&S processes in fresh produce operations.

Purpose: Evaluate current C&S practices in fresh produce operations to reduce microbial and organic loads on FCS.

Methods: Facilities with different C&S procedures were visited on a single operational day: onion (OPH; dry cleaning only), berry (BPH; wet cleaning), blueberry harvest contractor (BHC; cleaning+sanitizing), and two mixed vegetable (MVPH1, rinsing+sanitizing; MVPH2, cleaning+sanitizing). FCS (n=25 to 50) were sampled during production and after C&S. Sponge samples (3M) were analyzed for microbial load on tryptic soy agar (37°C, 24 h). Organic loads were assessed with Hygiena UltraSnap system.

Results: Dry cleaning at OPH did not reduce bacteria on conveyor belts (4.9±0.7 before vs. 5.1±0.4 log CFU/100 cm^2 after cleaning), and high soil levels prevented accurate measurement of organic load. Wet cleaning of plastic trays at BPH significantly reduced (*P*<0.05; t-test) microbial (from 3.1±0.9 to 2.5±0.7 log CFU/100 cm^2) and organic loads (618±616 to 158±133 RLU/100 cm^2). C&S procedures for harvest buckets at BHC significantly reduced bacteria from 3.8±0.5 to 1.1±0.4 log CFU/100 cm^2 (*P*<0.05), while organic load was low before and after cleaning (138±220 vs. 92±62 RLU /100 cm^2). Microbial and organic loads on conveyor belts (MVPH2) ranged from 5.0±1.0 to 4.3±0.3 log CFU/100 cm^2 and 817±1922 to 1231±1819 RLU/100 cm^2 before and after C&S, respectively. Water rinse followed by bleach (MVPH1) significantly reduced microbial and organic loads on stainless-steel surfaces from 3.2±0.7 to 1.4±0.2 log CFU/100 cm^2 and 1038±811 to 106±54 RLU/100 cm^2 (*P*<0.05).

Significance: Wet C&S procedures in fresh produce operations were effective at reducing microbial and organic loads on FCS. Dry cleaning in OPK was ineffective at reducing microbial load, and high soil levels interfered with luminescence.

T5-01 Genomic Insights into the Fitness and Ability of Shiga Toxigenic *Escherichia coli* to Form Biofilms and to Persist in the Food Processing Environment

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Introduction: The attachment and survival of microorganisms to food contact surfaces and ability to survive disinfectants are major causes of food adulteration.

Purpose: To perform a genomics comparison of STEC strains that differed in their ability to form biofilm and survive sanitation procedures

Methods: Biofilm formation ability was tested at 25°C using the crystal violet technique (n=385). STEC susceptibility to the following biocides was evaluated (n=720): quats (550ppm), sodium hypochlorite (1200 ppm), sodium hydroxide (50ppm), hydrogen peroxide (250ppm), hydrogen peroxide+acetic+peroxyacetic acid (250 ppm) and BioDestroy® (600ppm). Whole genome sequencing was performed following the illumina MiSeq procedure. Genome assembly was done using SPAdes; annotation was done using RAST tool kit. Genomes were compared using the comparative system's service (BV-BRC). *E. coli* O157:H7 str. Sakai was included as a reference genome. Four STEC strains were included in this study, three strong biofilm formers: O157:H7-R508, O157-1934, O103:H2 and one weak biofilm former O145:H2.

Results: All STEC biofilms resisted sodium hypochlorite and were susceptible to the rest of the sanitizers (*P*<.0001). Comparative genomics showed a pan-genome of 5495 protein families, a core genome of 3833 genes and 1662 accessory genes. Strain O145:H2, a weak biofilm former, lacks some genes associated with biofilm formation, such as secretion systems proteins, including T3SS (YscF, YscQ, YopD, EprH), T6SS (ImpABCGHIJK, VasBCDE, TssE, IcmF, Clpv). Other missing genes included fimbrial-like protein (YraK,YraH) and minor fimbrial subunit (StfF, StfE). All the strains carry genes associated with hypochlorite resistance, specifically YdhC (inner membrane transport protein) and multidrug efflux system EmrAB-OMF.

Significance: Some of the missing genes in the weak biofilm former strain might play an important role on biofilm formation. Biofilm formation and resistance to sanitizers are likely providing STEC strains with an adaptive advantage to persist in food processing environments.

T5-02 Genome-Wide Association Study of *Escherichia coli* Isolates from Food and Clinical Sources Identifies Genetic Markers Associated with *Shigella* Inhibition

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Introduction: Current microbiological methods for detection of *Shigella* in foods are unreliable and can be impacted by the presence of interfering organisms in enrichment cultures, including *E. coli* that are able to produce *Shigella*-inhibiting bacteriocins, leading to false-negative results in food safety surveillance.

Purpose: The goal of this study was to use genome-wide association studies (GWAS) to identify genomic markers in *E. coli* that are associated with *Shigella* inhibition.

Methods: Cell-free extracts from 462 *E. coli* isolates were spotted onto soft agar containing *S. sonnei* to identify isolates capable of interfering growth of *S. sonnei*. Whole genome sequences were generated for *E. coli* isolates, and genes associated with *Shigella*-inhibiting *E. coli* were identified by GWAS using Prokka for annotation and Roary for pan-genome analysis of draft genomes. Concordance between the presence/absence of genes and the observed *Shigella* inhibitory phenotypes was evaluated using Scoary. Bacteriocins were identified in genomes by aligning open reading frames to the BAGEL4 databases.

Results: S. sonnei inhibition was produced by cell-free extracts from 24.5% (113/462) of the *E. coli* tested. Most of the inhibitory isolates were recovered from beef (52/113), poultry (26/113) and human clinical samples (23/113). Based on the GWAS, 319 putative genes were significantly associated with *Shigella* inhibiting *E. coli* (Bonferroni-corrected *p*-value<0.05), including colicins E5 and 10, along with genes encoding colicin immunity proteins and lysis proteins, and virulence factors such as the Shiga toxin type 1 gene (*stx1*).

Significance: The genes identified in this study could be used as targets for developing improved approaches for detecting *Shigella* from foods that mitigate the impact of interfering *E. coli* in enrichment cultures, thereby reducing false negatives in food testing programs. Furthermore, the colicins identified in this study may also have therapeutic implications, as they have the potential to inhibit the growth of Shigella in various settings.

T5-03 Subtyping Evaluation of *Salmonella* Enteritidis Using Singlenucleotide Polymorphism and Core Genome Multilocussequence Typing with Nanopore Reads

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Introduction: Whole-genome sequencing (WGS) for public health surveillance and epidemiological investigation of foodborne pathogens predominantly relies on sequencing platforms that generate short reads. Continuous improvement of long-read nanopore sequencing, such as Oxford nanopore technologies (ONT), presents a potential for leveraging multiple advantages of the technology in public health and food industry settings, including rapid turnaround and onsite applicability in addition to superior read length.

Purpose: Using an established cohort of *Salmonella* Enteritidis isolates for subtyping evaluation, we assessed the technical readiness of nanopore long read sequencing for single nucleotide polymorphism (SNP) analysis and core-genome multilocus sequence typing (cgMLST) of a major foodborne pathogen.

Methods: All *Salmonella* strains were sequenced on a GridION sequencer and a NextSeq2000 sequencer. SNP and cgMLST were performed using ONT and Illumina sequences respectively, and statistical analysis was conducted to compare the difference between ONT and Illumina.

Results: By multiplexing three isolates per flow cell, we generated sufficient sequencing depths in ,7 h of sequencing for robust subtyping. SNP calls by ONT and Illumina reads were highly concordant despite homopolymer errors in ONT reads (R9.4.1 chemistry). In silico correction of such errors allowed accurate allelic calling for cgMLST and allelic difference measurements to facilitate heuristic detection of outbreak isolates.

Significance: Evaluation, standardization, and implementation of the ONT approach to WGS-based, strain-level subtyping is challenging, in part due to its relatively high base-calling error rates and frequent iterations of sequencing chemistry and bioinformatic analytics. Our study established a baseline for the continuously evolving nanopore technology as a viable solution to high-quality subtyping of *Salmonella,* delivering comparable subtyping performance when used standalone or together with short-read platforms. This study paves the way for evaluating and optimizing the logistics of implementing the ONT approach for foodborne pathogen surveillance in specific settings.

T5-04 Genomic Structure and Diversity of *SPV* Virulence Plasmids and Hybrid MDR-*SPV* Virulence Plasmids in *Salmonella*

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Introduction: Here we evaluate the composition of 38 *spv* virulence operon encoding plasmids (*spv*-plasmids) recovered from *Salmonella* isolated from retail meats, food animal cecal samples and diseased animals in the US.

Purpose: The purpose of this study was to evaluate the genetic diversity and structure of *spv*-plasmids carried by multiple *Salmonella* serovars with diverse AMR profiles.

Methods: Closed sequences of 38 *Salmonella spv*-plasmids from eight *Salmonella* serovars were evaluated for AMR genes, virulence genes and plasmid Inc type using AMRFinder-plus and PlasmidFinder. Plasmids were grouped by genomic similarity through hierarchical clustering of plasmid pangenomic loci identified by Roary and piggy.

Results: Plasmids were clustered into 10 groups, labeled A through J, containing 1-15 plasmids with a maximum of 5 plasmid replicon markers per plasmid. Plasmid groups A, B, C, D and G contained no AMR genes. Group A *spv*-plasmids (IncFII(S)/IncFIB(S)) were found in Typhimurium, Typhimuirum(O5-), Cholerasuis, and I4,[5],12:i:-. Group B, C and D *spv*-plasmids (IncFII(S)/IncFIB(S)) were recovered from Enteritidis, Cholerasuis and Typhimurium, respectively. Group G *spv*-plasmids (IncFII(S)/IncX1_1) were exclusive to serovar Dublin. The remaining plasmid groups had unique AMR profiles. Group E *spv*-plasmids (IncFII(S)/IncFIB(AP001918)) from Typhimurium(O5-) contained *aph(3')-Ia*, bla_{TEM-1} and *sul1*. Group F *spv*-plasmids (IncFII(S)/IncFIB(S)/IncHI2A) from Cholerasuis and Typhimurium(O5-) encoded *aph(3")-Ib, aph(6)-Id*, bla_{TEM-1} and *tet(B)*. The remaining plasmid groups (H, I, J) were only identified in *S.* Dublin. Group H *spv*-plasmids (IncFIB(S)/IncX1_1/IncC) contained *aph(3")-Ib, aph(3')-Ia, aph(6)-Id*, bla_{CMY-2}, *floR, sul2* and *tet(A)*. Group I *spv*-plasmids (IncFIA/IncC) carried *aph(3")-Ib*, *aph(3')-Ia*, *aph(6)-Id*, bla_{TEM-1}, *floR*, *sul2* and *tet(A)*. The group J *spv*-plasmid (IncFII(S)/IncX1_1/IncFIB(AP001918)/IncFIA/IncC) carried *aadA12*, *aph(3")-Ib*, *aph(3')-Ia*, *aph(6)-Id*, bla_{CMY}, bla_{CMY-2}, bla_{TEM-1}, *floR*, *sul1* and *tet(A)*.

Significance: Our findings show that the great diversity of *Salmonella spv*-plasmids follows patterns of serotype, AMR content and plasmid Inc type. Evidence of hybrid AMR-*spv*-plasmids present the potential for enhanced transmissibility of virulent MDR *Salmonella* strains and a significant impact on public health.

T5-05 Omics Techniques Application in Classification of Foodborne Pathogens Response to Antimicrobials Treatments

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Introduction: To inhibit the growth of pathogenic microorganisms in food, antimicrobial agents have been in use for a long time as secondary inhibitors along with the traditional food preservation techniques. Recent developments in "Omics" technology show promising application in food safety to improve pathogens detection, classification, characterization, and its response to stress at various environmental conditions and antimicrobials, new antimicrobial developments etc.

Purpose: To review the Omics techniques; transcriptomics, proteomics, and metabolomics in assessing the foodborne pathogens response to antimicrobial treatments and discovering new antimicrobial food ingredients.

Methods: Omics techniques provide deep understanding of fundamental characteristics of foodborne pathogens like adaptation, virulence, antimicrobial resistance, and resistance to environmental stress factors. In the past decade, many studies have assessed the application of Omics techniques in evaluating the stress and responses of bacteria at various conditions, like antimicrobials, water activity, pH, temperature etc.; this review is narrowed to selected foodborne pathogens response and stress against antimicrobials. Omics techniques applied to study the response to antimicrobials and other preservation techniques of major foodborne pathogens like *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* research were reviewed here.

Results: Investigation on *Listeria monocytogenes* exposure to lactate and diacetate antimicrobials using transcriptomics techniques showed that the synergistic effect of these ingredients shifted the fermentation pathways to produce acetoin, which acidify the cytoplasm, and produce less energy and reduce the growth rate of *Listeria monocytogenes.* Omics studies assessed different pathogens in various food matrices showed that there is an increase in expression of stress responses, and it has potential impact to pathogen resistance to the antimicrobial treatments and the pertaining environmental changes in the food matrices.

Significance: Transcriptomics is useful in assessing the pathogens' response to antimicrobials and its effect at molecular level to improve the food safety and further applied to developing new effective antimicrobials.

T5-06 Genomic Analysis of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus suci* Reveals Genetic Differences That Could Contribute to Differences in Spoilage Potential

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❖ ***Developing Scientist Entrant***

Introduction: *Alicyclobacillus acidoterrestris* and *A. suci* produce the spoilage metabolite guaiacol and are closely related, but there is a gap in understanding about the genetic differences between these species.

Purpose: The purpose of this study was to: (1) identify genes unique to either *A. acidoterrestris* or *A. suci* and (2) assess potential single locus PCR targets appropriate for differentiating isolates of these species.

Methods: A genome-wide association study (GWAS) was performed by annotating 34 *A. suci* and *A. acidoterrestris* assemblies using prokka v1.14.5. Pangenome analysis was performed using panaroo v1.3.0. The genes identified by Scoary v1.6.16. with p-values $<4.5\times10^{-10}$ were assigned to Clusters of Orthologous Gene (COG) categories using EggNOG-mapper v2. For the second objective, the 16S, *gyrB*, and *rpoB* gene sequences were extracted from the WGS data of 31 *Alicyclobacillus* representing 9 species using a custom BLAST search. RAxML phylogenetic trees were constructed for each gene using 1,000 bootstrap replicates.

Results: The GWAS identified 194 genes unique to *A. acidoterrestris* and 161 genes unique to *A. suci*. The COG analysis of these genes found they were involved in amino acid, ion, and carbohydrate transport and metabolism; transcription; and energy. *A. suci* was found to have an additional, unique gene involved in defense and *A. acidoterrestris* was found to have additional, unique genes involved in cell cycle (n=2) and motility (n=1). The phylogenies generated from the *rpoB* and 16S gene sequences showed clear clustering by species. By contrast, the phylogeny generated from the *gyrB* gene sequence did not well differentiate between *A. acidoterrestris* and *A. suci.*

Significance: The genes unique to either *A. acidoterrestris* or *A. suci* reveal patterns in metabolite transfer and metabolism, which has implications for spoilage mitigation strategies. Single locus PCR targeting *rpoB* and 16S can be used to differentiate *A. acidoterrestris* and *A. suci*.

T5-07 Characterization of Low-Moisture Food Persistent Bacterial Populations and Impacts of Nutrient Type, Moisture Ratio, and Relative Humidity

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Introduction: Minute amounts of moisture introduced to dry processing plants may be a source of pathogen contamination that result in persistent bacterial populations (PBP) and subsequent recalls of low-moisture foods (LMF).

Purpose: The impacts of moisture, nutrient source, and relative humidity (RH) on the formation of LMF PBPs on stainless steel were characterized. Methods for creating an LMF PBP were developed and compared for future work focused on inactivation of LMF PBPs.

Methods: Several methods were tested to determine a sound and reproducible method to form LMF PBPs. One method applied a pre-made slurry of powder with the liquid *Salmonella* Tennessee culture (14 g/10 ml). The second method sequentially applied 0.5 ml of *Salmonella* Tennessee and nonfat dried milk powder (NFDM) or silicon dioxide (SiO_2) (0.1 or 0.4 g) to stainless steel coupons. Coupons were stored at 22 to 24°C at predetermined 30 to70% RH, and *Salmonella* was enumerated at predetermined dates for 0 thru 28 days.

Results: *Salmonella* persisted on stainless steel coupons as an LMF PBP. Survival was impacted by moisture, nutrient type, and nutrient-to-moisture ratio. The *Salmonella populations* in NFDM was reduced by 2.4 and 2.9 log CFU/cm^2 at 30% and 44% RH, respectively, by inoculating slurries. In contrast, over 7 days, the *Salmonella* populations in NFDM (0.5 ml: 0.4 g) decreased by 1.4 and 3.9 log CFU/cm^2 at 50% and 70% RH, respectively. Over 28 days, the *Salmonella* populations in NFDM and SiO_2 (0.5 ml:0.4 g) reduced by 0.9 and 2.4 log CFU/cm^2 at 59% RH.

Significance: Determining effects of nutrient type, moisture/nutrient ratio, and relative humidity have on survival of pathogens in LMF PBPs will help industry develop the best approaches for cleaning and sanitation of LMF processing environments. Developing a standard LMF PBP method will allow for next steps of research to investigate eliminating LMF PBPs.

T5-08 Survival of *Salmonella enterica* Serovar Agona in Low-Moisture Environment

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Introduction: *Salmonella enterica* serovar Agona caused recurrent multistate outbreaks associated with cereal between 1998 and 2008, highlighting the persistence of *Salmonella* over time in food processing facilities. Understanding the survivability and physiology of this pathogen in low moisture food (LMF) and low-moisture environments is necessary for developing future intervention strategies.

Purpose: To determine the survivability and changes in morphology of *S.* Agona strain in low moisture environment and in LMF.

Methods: *Salmonella* Agona strains (CFSAN 000471 and 000477) linked to the two outbreaks of salmonellosis separated by ten years was selected for the study. 100µl suspension with a concentration of ~10^{10} CFU/ml was inoculated in 10g of rice cereals. Three replications of inoculated and uninoculated

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(control) cereals with each strain were subjected to desiccation stress (aw ≤ 0.25) for 30 days at room temperature (25ºC). Inoculated cereal was sampled for 15 timepoints post-inoculation. Cell morphology was examined in the desiccation state using Confocal Laser Scanning Microscopy (CLSM).

Results: Population levels of *Salmonella* were examined from inoculated cereals that underwent desiccation stress for 30 days. Right after inoculation, approximately 9 log CFU/g *Salmonella* cells were recovered. Population of *Salmonella* was then gradually decreased by 2 log CFU/g within 8 hours of drying (*P*>0.05). However, after initial reduction, *Salmonella* population remained at ~7to 8 log CFU/g for 30 days. A tailing effect in the survival curve of *S*. Agona was observed indicating the existence of a resistant subpopulation within the main population. Changes in cell morphology were observed under desiccation stress.

Significance: These data suggest that very low humidity provides favorable environment for *Salmonella* Agona survival on cereal, suggesting the importance of proper sanitization for low moisture food processing environment.

T5-09 Inoculum Growth Method Impacts the Survival Kinetics of *Salmonella* and Shiga-Toxin Producing *Escherichia coli* inoculated onto Wheat Grain

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❖ ***Developing Scientist Entrant***

Introduction: Outbreaks in low moisture foods increased interest in evaluating *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) survival kinetics in low water activity environments. Studies have shown inoculum prepared with the surface growth impacted the population stability and inactivation kinetics of *Salmonella*.

Purpose: Quantify and model the survival kinetics of *Salmonella* and STEC on wheat grain using four different inoculum growth methods.

Methods: Three strains of *Salmonella* (serovars Agona, Enteritidis, and Mbandaka) and three strains of STEC (serotypes O157:NM, O26:H11, and O121:H19) were inoculated onto wheat at 8 log CFU/g using different inoculum growth methods: aerobic liquid growth in LB (L+), aerobic and anaerobic surface growth on LB agar (S+/S-), and aerobic surface growth on tryptic soy +1% glucose agar for acid-adaptation (SA+). After inoculation, a_w was adjusted to 0.45. Bacterial populations were quantified by plate count after inoculation, after water activity equilibration (Day 2), and during storage at 20°C, 65% RH for up to six months. Weibull-Mafart's model was used to fit the inactivation of *Salmonella* and STEC populations after water activity equilibration.

Results: After equilibration, a_w of wheat grain was 0.47±0.02 and remained stable during storage. Differences in inactivation rates were found to vary by inoculum growth method. The first decimal reduction ranges from 14.2 to 34.5 days for L+, 26.0 to 73.6 days for S+, 20.1 to 60.7 days for S-, and 17.4 to 57.2 days for SA+. Pathogens grown using the L+ method had significantly lower survival rates on wheat compared to those prepared by S+ and S-. *S.* Enteritidis survived significantly better than *S.* Agona and STEC on wheat when the inoculum was prepared with the S+ and S- methods.

Significance: Survival kinetics of *Salmonella* and STEC on wheat grains are impacted by the inoculum growth method; differences between species and within species among isolates were also observed.

T5-10 Developing Predictable Thermal Treatments for Control of *Salmonella* in Low-Moisture Foods Using Kinetic Models That Include Water Activity as a Key Parameter

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Introduction: It is well known that pathogens are difficult to control in low-moisture foods. The wide range of low-moisture food products produced by different processing operations presents a major challenge for the food industry in coming up with a unified approach to develop predictable and effective thermal treatments. We have recently established quantitative relationships between dynamically changing water activity (with temperature) in low-moisture food with log-reduction value (*D*-value) of several bacterial pathogens, including *Salmonella,* in different matrices over a wide temperature range. Guided by such knowledge, we were able to systematically develop predictable thermal treatments for different applications. In this presentation, we will present three case studies in which predictable thermal treatments for the control of *Salmonella* were developed and validated with the help of thermal death kinetic data that include the influence of food water activity or environmental relative humidity (RH).

Purpose: Demonstrate the use of kinetic models, that included water activity as a parameter, in developing and validating three different thermal treatments for pathogen control in low-moisture foods.

Methods: Pilot-scale thermal treatments for control of *Salmonella* were developed for the three cases: 1) treating wheat flour in sealed packages using RF bulk heating; 2) treating peppercorn using controlled high relative humidity hot air; 3) using a high relative humidity, relative short-time treatment step before applying conventional drying. The treatments were developed based on kinetic models that included water activity as a key process parameter and validated in inoculation tests.

Results: The results indicate that in all three cases, the thermal inactivation of *Salmonella* was successfully predicted with a model using three parameters: temperature, RH (or high-temperature water activity of the food), and time.

Significance: This study has demonstrated the feasibility of using kinetic models that include water activity as a key parameter to develop predictable pasteurization treatments for different low-moisture food commodities.

T5-11 Optimization of Vaporized Hydrogen Peroxide Inactivation of *Salmonella* in Dried Basil Leaves by Central Composite Design

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❖ ***Developing Scientist Entrant***

Introduction: Recent foodborne illness outbreaks associated with the consumption of low moisture foods such as spices and herbs have emphasized concerns over microbial food safety.

Purpose: The objective of this study is to investigate the efficacy of vaporized hydrogen peroxide (H_2O_2) in inactivating *Salmonella enterica* in dried basil leaves immediately after processing and during storage post H_2O_2 treatment. *Enterococcus faecium* NRRL B-2354 was evaluated for its suitability as a surrogate of *Salmonella*.

Methods: Central composite design was used for optimizing the H_2O_2 processing parameters at two temperatures (32, 35 °C), and variables such as injection rate (2-6 g/min), gassing time (4-55 min), dwell time (1-30 min) with five levels for *Salmonella* and *E. faecium* inactivation in dried basil leaves. Bacterial reduction was also evaluated during storage for a week after H_2O_2 treatment.

Results: Maximum average log reductions of *Salmonella* (2.65 log CFU/g) and *E. faecium* (4.45 log CFU/g) were achieved when samples were treated at an injection rate of 4 g/min for 55 min followed by dwell for 15 min (CTP=1710 ppm-h). *E. faecium* was not a suitable surrogate for H_2O_2 in dried basil leaves. Due to a smaller range of response variable (log reduction), the response surface models did not perform well. Though H_2O_2 treatment did not provide the desired log reduction immediately, storage after processing significantly reduced *Salmonella*. Desired 5-log reduction was achieved in the treated samples after 7 days of storage at ambient conditions after H_2O_2 treatment at 6 g/min for 30 min. A significant difference was observed in the color value of H_2O_2 treated and untreated samples. However, the H_2O_2 treatment did not significantly impact the total phenolics and antioxidant activity of dried basil leaves.

Significance: Extended storage (7 days) post H_2O_2 treatment enhanced microbial inactivation and can be considered to design decontamination strategies for spices and herbs. This treatment could be used by industries to improve the safety of dried basil leaves.

T5-12 *Salmonella* Public Health Challenge and Its Near-Zero Detection Paradox in Low-Water Activity Food: A Linear Mixed Effects Modelling of 9656 Flours

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Introduction: *Salmonella* outbreaks linked with different serovars in low water activity foods such as flours has increased globally. But continuous monitoring efforts of *Salmonella* in flour though limited usually returns nil or near-zero detection. Hence, it is of public health interest to unravel this contradiction.

Purpose: This study aimed to determine potential factors that impact accurate detection/prevalence of *Salmonella* in 9656 flour samples.

Methods: *Salmonella-flour* specific data mined from five mega-databases (>40 linked repositories) using all-inclusive algorithms were logit-standardised and fitted to a linear mixed-effects model (LMM). The LMM was further examined for robustness via leave-one-out cross-validation. A mixed-effects univariate/multivariate meta-regression accompanied with 1000 permutation test was used to assay the influence effects of variables.

Results: The global prevalence of *Salmonella* in flours was 1.51% [95%CI: 1.29 tp 1.78] with a prediction interval of 0.00 to 61.52%. The cross-validation yielded 1.09% [0.89 to 1.32]. The regional prevalence of *Salmonella* in flour was not significantly different (*P*=0.66) with the highest from Asia (4.94% [0.41 to 39.67]), followed by North America (1.20% [0.91 to 1.58]), South America (0.89% [0.00 to 99.64]), 0.00% [0.00 to 100.00] in Africa, Australia, and Europe. Method, sample size (n), continent, and N+method accounted for 0.33% ($F_{1,21}$ 0.0074, *P*=0.93), 9.29% ($F_{1,21}$ = 0.87, p = 0.37), 87.63% ($F_{5,17}$=8.04, *P*=0.16), and 12.70% ($_{F2,20}$=0.51, *P*=0.63) variance respectively, on the detection/prevalence of *Salmonella* in the flours. At least, a three-factors multivariate of continent, flour type, n, and method accounted for 100% variance in *Salmonella* detection with a significant moderator influence.

Significance: This study concluded that sample size, method, continent (probably climate and human development index, etc.), and their interaction affect the detection of *Salmonella* in flours. It is recommended that surveillance efforts with improved methodological approach for detection of *Salmonella* in flours be strengthened across regions.

T6-01 Machine Learning, AI, and Confirmation Bias in Crowdsourced Foodborne Illness Reporting

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Introduction: Crowdsourcing is an emerging source of data for foodborne illness investigation. Valuable signals exist in crowdsourced data, however interpretation can be complicated by confirmation bias, or the tendency for evidence to be used in a manner that confirms or supports a particular view or hypothesis.

Purpose: Using two case studies as examples, identify considerations when analyzing crowdsourced data, with a focus on handling confirmation bias.

Methods: A sample of 8,300 crowdsourced reports (2018 thru 2022) of potential food-associated illnesses from iwaspoisoned.com, were reviewed. Example 1 consisted of 7,660 complaints (associated with 9,156 illnesses; reported Jan 1, 2018, thru Dec 31, 2022) about gastrointestinal illness (GI) seemingly associated with a single dry cereal product brand. Example 2 included an examination of 640 complaints (845 illnesses; reported Jan 1 thru Dec 31, 2018) of potential GI linked to a restaurant chain in Ohio (United States). These datasets were analyzed for anomalies using machine learning and cluster analysis, which were then further processed to identify potential confirmation bias by identifying potential sources of bias.

Results: In Example 1, a four-month-long national investigation failed to identify the causative agent. For Example 2, a two-month long investigation by state and federal partners revealed Clostridium perfringens as the cause of the illnesses. The website iwaspoisoned.com provided signals, complainant contact information and report data which aided in the epidemiological investigations for both examples. Confirmation bias was present in both examples, primarily due to news media coverage, and measures to address this were evaluated by examining the dates of media coverage and the timestamps of the user reports.

Significance: The examples demonstrate that the strength of a 'valid' signal obtained by crowdsourcing can vary greatly and can depend on the context, and confirmation bias must be considered, especially in cases with media coverage.

T6-02 Predicting *Vibrio parahaemolyticus* Concentration in Seawater and Oysters Using Machine Learning

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❖ ***Developing Scientist Entrant***

Introduction: *Vibrio parahaemolyticus* is a naturally occurring halophilic bacterium mainly found in the estuarine environments and transmitted to human through the consumption of raw or mishandled seafood, especially oysters. The increase in illnesses and outbreaks caused by this bacterium makes it a global concern.

Purpose: The purpose of this study was to determine the efficacy of different machine learning algorithms in investigating the effects of environmental parameters on *V. parahaemolyticus* concentration in seawater and oysters.

Methods: *V. parahaemolyticus* concentration in seawater and oyster samples collected from Delaware and Maryland, and environmental parameters during the time of collection were used to build the predictive models. Different machine learning algorithms (random forest (RF), support vector machine (SVM), elastic net, neural network (NN), k-nearest neighbors (KNN), and extreme gradient boosting (XGB)) were compared to identify the best fit based on accuracy statistics. The SHapley Additive exPlanations (SHAP) method was subsequently used to analyze the relative importance of environmental variables in predicting *V. parahaemolyticus* concentration.

Results: RF had the best regression statistics (R^2=0.75) among all the machine learning methods we used. According to SHAP analysis, the concentration of chlorophyll *a*, followed by turbidity and conductivity, were ranked as the top three most important variables contributing to the levels of *V. parahaemolyticus* in seawater samples.

Significance: Based on the results, machine learning is a good approach at predicting the concentration of *V. parahaemolyticus* based on environmental parameters. The identified important variables in this study could help manage the risk associated with *V. parahaemolyticus*, thereby reducing its public health burden.

T6-03 Applications of Multispectral Imaging (MSI) Coupled with Machine Learning for the Evaluation of Food Microbiological Quality and Authenticity

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Introduction: Multispectral imaging in the food industry has been proven to be extremely valuable since it can be used to evaluate several parameters.

Purpose: To estimate microbial quality and food fraud of several foodstuffs by implementing Multispectral Imaging (MSI) in tandem with different regression/classification models.

Methods: MSI analysis was performed using a VideometerLab instrument (Videometer A/S, Videometer, Herlev, Denmark) for more than 8,000 food samples. In brief, various animal (4400) and plant (1400) origin food commodities were analyzed microbiologically (total aerobic counts (TAC)), whereas about 2000 samples were collected covering different food fraud scenarios, such as mislabeling and adulteration. Subsequently partial least square regression (PLS-R), support vector machines (SVM), tree-based algorithms applied on data for feature selection and the development of regression/classification models. The root mean squared error (RMSE), coefficient of determination (R^2) and accuracy score of the test set or external validation were used for the evaluation of models' performance.

Results: Several algorithms e.g., SVM, Random Forest (RF) and PLS-R were used. In fact, the acquired measurements in tandem with SVM provided 90% accuracy scores for the prediction of different levels of pork in chicken meat and for the discrimination of fresh from frozen chicken thigh fillets. Also, the presence of bovine offal in beef was successfully detected. However, the discrimination of adulterated cooked beef samples was poor and further analysis is needed. In the case of discrimination of seabass and seabream fish the accuracy was >93% using RF. Regarding microbial counts (TAC) the analysis with PLS-R was found to be satisfactory with RMSE of the test set being <0.80 log CFU/g for chicken and beef. This was also the case in the estimation of microbial populations in brown edible seaweed (R^2, 0.80; RMSE, 0.90).

Significance: MSI data coupled with machine learning algorithms exhibit potential towards efficient detection of food fraud and microbial counts estimation in various foodstuffs. This work has been funded by the project DiTECT (861915).

T6-04 Validation of a Competition and Dynamic Model for *Salmonella* Growth in Raw Ground Pork during Temperature Abuse (10 to 40°C)

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Introduction: Pork is responsible for 8 to 13% of over one million annual foodborne salmonellosis cases in the US. To predict the growth of *Salmonella* more realistically in pork, it is essential to validate competition models incorporating background microbiota.

Purpose: To validate a competition and dynamic model for the growth of *Salmonella* in raw ground pork during temperature abuse.

Methods: A five-strain cocktail of *Salmonella* (3 to 4 log CFU/g) was inoculated into raw ground pork at two fat levels (ca. 5 and 25%). Five-gram inoculated pouches were submerged in water baths (10, 15, 20, 25, 30, and 40 °C) for 25 to 440 h. Cells were recovered on XLD agar for *Salmonella* and 3M Aerobic plate count (APC) a. The Jameson-No lag Buchanan model was fitted to the experimental data. Maximum growth rates (μ_max) were modeled as a function of temperature using a Cardinal parameter equation. The differential form of the Baranyi model with the Cardinal parameter equation was solved numerically using fourth order Runge-Kutta method in MicroRisk®. The dynamic model was validated using sinusoidal dynamic temperature profiles. Root mean squared error (RMSE) and Acceptable Prediction Zone (APZ) method were used to evaluate the model performance.

Results: The competition model was well fitted to the experimental data having 92% (496/540) residual errors within the desired APZ. The and the maximum population density were not different for two fat levels. The theoretical minimum and optimum growth temperature estimated by the Cardinal parameter model was 5.5 °C and 35°C, respectively. The simulated dynamic model showed good performance (pAPZ=0.96) in the prediction of the sinusoidal temperature of the experimental data.

Significance: Industry and regulators can use the results of the validated models to develop appropriate risk assessment and mitigation strategies to improve the microbiological safety of raw pork products.

T6-05 Identifying Stress Response Signatures in *Salmonella enterica* Isolates Using Machine Learning and Transcriptomics Data

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Introduction: Recent years have seen major advances in using '*omics*' data to characterize the molecular basis of foodborne pathogen behavior to improve food safety. Moreover, identifying the genetic and phenotypic bases of pathogen behavior under infection and food environmental conditions can help improve the accuracy of microbial predictive models, specifically in pathogens with high intra-species diversity, such as *Salmonella enterica*.

Purpose: Survival- and stress response-related signatures of *Salmonella enterica* were characterized using machine learning-based analysis of transcriptomic (RNA-Seq) data.

Methods: RNA-Seq transcriptomics data for *Salmonella enterica* serovars Typhimurium and Enteritidis subjected to food environment-related stressors was obtained from public repositories and prior *in vitro* transcriptomics studies with simulated bacterial stress conditions. The efficacy of random forest (RF), support vector machine (SVM), Logit Boost, and Elastic Net (EN) classification algorithms to classify *Salmonella* isolates into groups based on differential gene expression signatures was tested. Elastic Net regression was further used to identify the most impactful genes associated with *Salmonella* stress and survival response.

Results: The best performing model was identified using confusion matrix statistics and the area under the receiver operating characteristic curve (AUC-ROC). The best performing model was Elastic Net (AUC-ROC=0.72; classification accuracy=0.68). Elastic Net identified 35 genes that were important to the model, among which eight were identified as being statistically significant. Further regression analyzing for serovar-specific and stress response-specific signatures identified 29 and 31 genes significant for temperature and pH stress, respectively ($P<0.05$) in *Salmonella* Enteritidis.

Significance: Transcriptomics analyses help in characterizing gene expression under specific conditions encountered by pathogens, transcending gene presence/absence information. Using such data, machine learning-supported weighted regression predicts *Salmonella enterica* host environment-related stress with high accuracy. These models help reevaluate the overall survivability of bacteria in food systems. This could help in characterizing bacterial survival from farm-to-fork to help in revising current microbial risk assessments.

Technical

T6-06 A Novel Framework to Estimate *Salmonella* Dose-Responses Accounting for Genomic Serovar Virulence and Exposures from Food Sources

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Introduction: Quantitatively valuating microbial criteria (MC) that differentially target virulent serovars requires dose-response (DR) models that account for virulence differences.

Purpose: to develop a framework to derive DR functions for serovar groups based on genetic virulence factors amenable to non-typhoidal *Salmonella* (NTS) risk assessments.

Methods: We fitted an unsupervised random forest to group NTS serovars into higher virulence (HV) and lower virulence (LV) groups based on genetic virulence factors. We then estimated a beta-Poisson DR function for the HV serovars using a hierarchical Bayesian framework based on published outbreak data linked to HV strains. Using beef and poultry attributed salmonellosis as a model, we scaled up/down the DR model for the LV serovar groups via multipliers that adjusted for the relative risks of illness from food exposures to serovars from each group.

Results: 36,647 NTS strains submitted to NCBI were used to derive virulence groups. US regulatory isolates from beef (1,452) and poultry (13,537) products, together with 792 NTS outbreaks with an identified food source were used to calculate the multipliers. The proportion of HV serovars was 27.7% (95%CrI:18.3-38.8%) in beef and 33.4% (31.7-35.2%) in poultry samples. In contrast, HV serovars represented 67.6% (43.1-87.2%) of beef and 71.3% (57.8-82.8%) of poultry outbreaks. Weighting by consumption (34% beef, 66% poultry) resulted in a multiplier of 2.3(1.8-2.7) for HV serovars and 0.39 (0.24-0.56) for LV. Resulting DR functions exhibit large differences. For example, the probability of illness (P_{ill}) following consumption of an average of 10 CFU of LV serovars was 1.3% (0.4%-4.5%), whereas P_{ill} was 9.8% (4.0-25.5%), or 7.4 times larger for HV serovars. This ratio approaches the 2.3 multiplier at CFU/g > 10^9.

Significance: Our novel framework pairing genomics and DR modeling can be applied to any DR function or source of foodborne NTS. This is essential for risk assessments evaluating MC differentially targeting virulent NTS serovars.

T6-07 A Quantitative Microbiological Risk Assessment for Relative Impact of Peripheral Lymph Nodes on *Salmonella* Due to Consumption of Ground Beef in the U.S.

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Introduction: The inclusion of peripheral lymph nodes (PLNs) contaminated with *Salmonella* in fresh ground beef production may pose risks to public health. The adoption of PLN-targeting interventions heavily relies on a thorough assessment of their efficacies in achieving expected food safety and public health objectives, which requires a quantification of the relative contribution of PLNs to salmonellosis risks.

Purpose: Quantify the relative contribution of PLNs on salmonellosis risk associated with ground beef consumption using a quantitative microbial risk assessment (QMRA) approach.

Methods: A QMRA model in a processing-to-table continuum of ground beef processing was developed covering four modules from fabrication and trimming, grinding and partition, through transportation and storage, till preparation before consumption. The risk metric was measured as annual incidence cases of salmonellosis in 100,000 population in the U.S. Second-order Monte Carlo simulation using @Risk software was conducted to evaluate the influence of PLNs-related variables.

Results: The annual incidence of salmonellosis cases per 100,000 people in the U.S. attributable to ground beef consumption was estimated as 49.3 (90% CI: 45.2 to 53.4%) for cull cattle and 6.7% (90% CI: 6.1 to 7.2%) for fed cattle. Sensitivity analysis results showed cooking temperature was the most influential factor on reducing salmonellosis cases. According to relative impact results, PLNs-related factors had the lowest impact on the risk estimate, compared to other factors, i.e., non-PLN ones (e.g., the contamination originating from the carcass surface) and consumers-related ones (e.g., the food preparation and hygiene practices), indicating the removal of PLNs can be related to a reduced risk but may not be as effective as the other interventions.

Significance: The present study allows for the quantification of PLNs' contribution to *Salmonella* infections from ground beef consumption and offers the possibility to adopt PLN-related interventions by considering their effectiveness in public health protection and acceptance of implementation costs.

T6-08 A Novel Quantitative Microbial Risk Assessment Framework Incorporating Genomic Virulence to Assess the Public Health Impact of Alternative Microbial Criteria for *Salmonella* in Beef

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Introduction: Current US microbial criteria (MC) for beef treat all *Salmonella* serovars equally. Yet, as variation in *Salmonella* serovar virulence exists, MC increasing sampling while differentially targeting serovars in beef may be more efficient in reducing the burden of salmonellosis.

Purpose: To conduct a quantitative microbial risk assessment (QMRA) evaluating the public health impact of alternative MC for *Salmonella* in beef incorporating concentration, virulence, and increased sampling.

Methods: We developed a QMRA model to assess the impact on incidence of salmonellosis of different scenarios of MC based on proportion of production units - known as combos - tested (*Testp*), load thresholds per 325g samples (*LOD*), and differential targeting of the "higher" (HV) and "lower" (LV) virulence serovars. These MC scenarios were compared against a baseline with current FSIS testing schedule (*Testp*=0.02%) with all *Salmonella* treated as adulterant (*i.e.*, *LOD*=1 CFU/325g, all positive combos diverted). The QMRA incorporates a novel genomic method to group serovars by virulence, and group-specific *Salmonella* dose-response curves to reflect differences in HV and LV infectivity.

Results: Illness reductions >25% can be achieved for *Testp* >75%, and *LOD* of 1 or 10 CFUs/325g sample. For example, a mean reduction of 57% (95%PrI=47.6-63.5%) is predicted with *Testp*=100% at *LOD*=10 CFUs/325g. At high *Testp*, predicted illnesses were similar while the proportion of combos diverted was larger for LOD=1 *vs* LOD=10 CFUs/325g (e.g., 0.41% vs 0.07% with *Testp*=100%). MC targeting only HV serovars achieved a statistically equivalent illness reduction as MC targeting both HV and LV, but with less diverted combos. For example, only applying the LOD of 10 CFUs/325g to HV with *Testp*=100% led to a mean reduction of 50% (39.4-57.5%) and 0.02% of combos diverted.

Significance: Testing a high proportion of combos while targeting HV serovars at an LOD of ≤10CFUs/sample can significantly reduce human salmonellosis compared to the baseline FSIS testing schedule.

T6-09 Comparison of Source Attribution Methodologies for Human Campylobacteriosis

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Introduction: Different methodologies such as machine learning, network analysis and bayesian methods have proven valuable in microbial source attribution using the increasingly available and complex structured genome sequencing data (WGS)while inputting WGS outputs such as multilocus sequence typing (MLST), core genome multilocus sequence typing (cgMLST), K-mer, and single-nucleotide polymorphism.

Purpose: This study compares the performance of the different approaches for utilisation of WGS in source attribution to improve the resolution of source attribution models.

Methods: We compared the source attribution methodologies for different WGS data inputs cgMLST and K-mers (5-Mers and 7-Mers)) using machine learning, network analysis, and a bayesian approach using *Campylobacter* spp. as a case study.

Results: The network analysis algorithm had an accuracy value of 78.99% coherence source clustering and an F1- score value of 67%. However, the machine learning algorithm showed the highest accuracy (98% accuracy). Network analysis approach with 5mer attributed 965 cases while machine learning all 1224 human cases to their most probable animal sources. Poultry were the primary cause of human campylobacteriosis with an average percentage probability of attribution of 45.8% when bayesian approach was used based on 7-Mer as input and 65.4% for machine learning based on cgMLST data.

Significance: While the different source attribution methodologies based on WGS vary in performance, machine learning methods have the potential to utilize the increasingly available and complex structured WGS data for higher resolution surveillance and monitoring of *Campylobacter.* The models described can easily be adapted to other pathogens to prioritize and evaluate interventions.

Technical

T6-10 An Innovative Approach for Assessing Source Attribution of Foodborne Illnesses: Understanding the Risk to Inform Decision-Making

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Introduction: Foodborne illnesses represent significant public health concerns so it is essential to identify the causative pathogens and food sources to implement adequate control measures. Within this context, the Canadian Food Inspection Agency developed the Establishment-based Risk Assessment (ERA)-Food model that considers the type of food manufactured, to allocate inspection resources to the highest risk areas.

Purpose: As part of the model continuous update, a new approach for foodborne illnesses' source attribution was designed considering food availability and serving size consumed by Canadians as a measure of exposure. This helped experts compare the risk across commodities/sub-products for each pathogen using an equal available serving size.

Methods: Using an interactive questionnaire, two consultations involving 37 food safety experts were designed to determine the (1) relative contribution of 18 commodities to foodborne illnesses in Canada caused by 17 pathogens, and (2) source attribution at the sub-product level for 34 pathogen-commodity combinations responsible for the majority of the health burden. Weighted medians and 95% confidence intervals were calculated, along with the non-parametric Mann-Whitney U test to evaluate differences as a function of the experts' experience and work affiliation.

Results: Results identified the strongest contributors to Canadian foodborne illnesses with varying levels of certainty depending on the pathogen-food combination. Compared to previous studies, a greater number of yearly cases per available serving size of *Campylobacter* in poultry products (+17%), and *E. coli* O157 in beef products (+29%) were observed. Also, experts' estimates were not significantly impacted by their years of experience in food safety (i.e., <20 y vs ≥20 y) when attribution was done at the commodity level, but eight were significantly impacted ($P<0.05$) at the sub-product level (e.g., beef-*Listeria monocytogenes*).

Significance: Results of this study were incorporated into the ERA-Food model to improve its ability to prioritize and to efficiently allocate inspection resources based on risk.

T6-11 Performance Assessment of the Canadian Food Inspection Agency's Importer Risk Assessment Model: Application on Importers of Fruits and Vegetables (Fresh and Processed)

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Introduction: The Canadian Food Inspection Agency (CFIA) has developed an Importer Risk Assessment (IRA) model to assess the food safety risk of importers and proactively respond to emergent risks linked to food trade globalization.

Purpose: A performance assessment of the IRA model was conducted, testing the algorithm on importers of fruits and vegetables and evaluating the level of agreement between the assessment completed by senior inspectors and the risk results generated by the model.

Methods: A one-year importers' dataset related to the inherent and mitigation IRA risk factors was extracted in addition to compliance factors collected from CFIA databases, and used to run the algorithm. Thirty-two importers were randomly selected using the volume as a strata. Seventeen CFIA experts participated on a voluntary basis, and each of them categorized ten importers including two controls (low and high risk) into five risk categories. A Spearman correlation analysis was conducted on the inherent, mitigated, and final risk results, and a t-test was used to test the significance of difference between the correlations.

Results: Sixty-five percent of participating experts had over 10 years' experience in food safety and their background varied (i.e., 29% food science, 30% biology/microbiology, 6% animal science and veterinary). When assessing the final risk assessment done by the IRA model and the experts, a significant correlation was found (0.70, $P<0.05$). For the two remaining model's components (inherent and mitigated risk), results also showed a high (0.80, $P<0.05$) and moderate (0.53, $P<0.05$) positive correlation respectively.

Significance: These findings suggest an adequate performance of the CFIA's IRA model on assessing the risk of food importers. This tool will be implemented by the agency to guide the allocation of inspection resources.

Technical

T6-12 Qualitative Risk Assessment of Viable-but-Nonculturable *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium on Field-Grown Romaine Lettuce

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Introduction: Leafy green vegetables are recognized as the potential vehicles for foodborne pathogens such as *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium. Both bacteria can enter a viable-but-nonculturable (VBNC) state to evade the conventional culture-based detection and survive under unfavorable conditions, posing a great challenge to food safety. Risk assessment is an effective way to evaluate the potential threats of VBNC pathogenic bacteria on fresh produce.

Purpose: This study aimed to assess the risk of VBNC *E. coli* O157:H7 and *S.* Typhimurium on Romaine lettuce during pre-harvest, processing and storage stages.

Methods: Culturable and VBNC *E. coli* O157:H7 and *S.* Typhimurium were separately inoculated on Romaine lettuce grown in the field plots, and their amounts were monitored during 21-day post-inoculation, post-harvest processing and storage at 4°C and 12°C, respectively. Qualitative risk assessment was performed using a risk table method. The risk levels of the critical control points were assessed based on its probability of occurrence and severity.

Results: VBNC *E. coli* O157:H7 and *S.* Typhimurium showed high resistance to stresses and the ability to resuscitate during the field-grown stage, indicating a higher potential risk level compared to the culturable counterparts. Washing with chlorinated water after harvest effectively reduced the risks of these two pathogens from high to low level. Risk levels for both bacteria increased to medium level after one week storage and *E. coli* O157:H7 showed a higher risk level than *S.* Typhimurium. Storage at 12°C also increased the risk of both pathogens compared to that at 4°C.

Significance: The risk of VBNC *E. coli* O157:H7 and *S.* Typhimurium on field-grown Romaine lettuce was qualitatively evaluated, providing a better understanding of the threat of VBNC pathogens on fresh vegetables and useful guidance on effective control strategies.

T7-01 No-Enrichment *Listeria* spp. Detection Tool for Environmental Pathogen Monitoring

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Introduction: Environmental monitoring program (EMP) for *Listeria* can help track and mitigate sources of *L. monocytogenes* in the production environment and verify the effectiveness of control measures. An example is the novel *Listeria* Right Now (LRN) swab test, developed few years ago, which can sensitively detect *Listeria* spp. from environmental surfaces in less than an hour, without the need for an enrichment step. Sponges are more commonly employed for routine EMP because a larger surface area can be sampled and monitored.

Purpose: Compare the performance of the new isothermal ANSR LRN-Sponge Kit test system for EMP applications to a culture-based enrichment method.

Methods: *Listeria* along with 10 to 200 times higher background flora were inoculated on plastic or sealed concrete surfaces. Two sets of samples for each surface type were prepared for LRN-Sponge and culture methods, respectively. Each set of environmental samples included 20 replicate 4” by 4” surfaces inoculated with low-level *Listeria* for fractional positive result, along with five high-level and five uninoculated surface samples. LRN-Sponge samples were also tested by q-PCR assay for additional verification. Statistical model measuring differences of POD (dPOD) and 95% confidence-interval were calculated to compare different analytical methods.

Results: No significant difference was found between LRN-Sponge EMP Kit and the cultural method for detection of *Listeria* from the plastic environmental surfaces. For sealed concrete inoculated with high level of *Listeria*, the LRN-Sponge detection approach was more sensitive, detected 5/5 positive and culture method only detected 1/5 positive. When the LRN sponge samples were tested with the q-PCR assay, there was no significant difference between LRN-Sponge and q-PCR results.

Significance: No enrichment, sensitive and rapid detection in less than an hour is a major advancement in pathogen control, allowing personnel in food service, processing, or production settings to take immediate corrective action if *Listeria* spp. is found in the environment.

T7-02 Determination of Organic Contaminants in Food and Nutraceuticals Using Ultra-Performance Liquid Chromatography/Tandem Mass Spectrometry

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Introduction: Chemical contaminants and adulterants may exist in food and nutraceuticals. The combined approach of non-target screening and target analysis could identify and quantify emerging pollutants in food.

Purpose: This study determined 33 previously identified and prioritized organic chemicals in 23 food items and 12 prioritized chemicals in 19 nutraceuticals using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS).

Methods: 127 food samples were collected in Taiwan from February to March 2022. Homogenized 1-g samples were extracted with QuEChERS and cleaned up with PRiME HLB, and 33 analytes were separated on a Waters CORTECS C18 column using UPLC-MS/MS at electrospray ionization with isotope-dilution techniques. Analytes in nutraceutical samples were separated on a Lunar Omega C18 column with UniSpray ionization.

Results: Pesticides were found in vegetables generally lower than two ng/g, but pencycuron was up to 367 ng/g in bok choy. Abietic acid was detected in 7 of 32 seafood samples from 23.0 to 88.8 ng/g; ethoxyquin was seen in 2 of 5 salmon samples at 14.8 ng/g on average. Abietic acid (410 ng/g on average) was also observed in 5 of 6 pork liver samples. Organophosphate flame retardants were detected in 8 of 17 grains and root crops. Bis(2-ethylhexyl) adipate (DEHA), a new plasticizer, was found in 23 of 31 meat and poultry products ranging from 0.42 to 38.7 ng/g. Diazepam was detected in turtle deer drink and sesame seed extract from 10.5 to 32.1 ng/g, which were considered low health risks. The concentration of bis(2-ethylhexyl)phthalate (DEHP) in soy extract was 1.62 μg/g, and its daily intake exceeded the tolerable daily intake of the Taiwan FDA.

Significance: Most pesticide residuals in food complied with the MRL requirements. Industrial chemicals, feed additives, and human and veterinary drugs were the primary pollutants in seafood products. DEHP and other plasticizers warrant further study on the health risks of taking nutraceuticals.

T7-03 Development of a Mass Spectrometry Method for the Detection and Quantification of Peanut Protein in Processed Food Matrices

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Introduction: The food industry utilizes analytical methods to detect the unintentional presence of peanut in food products for regulatory purposes and peanut-allergic consumer safety. Current immunoassays have displayed decreased recovery and cross-reactivity issues in processed food matrices, but mass spectrometry (MS) methods may overcome these deficits.

Purpose: The objective of this work was to develop a sensitive and robust MS method to detect and quantify peanut protein in processed food matrices at relevant concentrations to protect the peanut-allergic population.

Methods: Incurred cookie and dark chocolate matrices were generated with peanut flour at various concentrations (0-5,000 ppm peanut protein). A quantitative MS method was developed using nine target peptides identified through a discovery-based target selection approach. Optimization of the method, including instrument parameters, liquid chromatography, and sample preparation, was pursued to increase sensitivity and address variability. The method was evaluated against incurred cookie (1.24 and 6.21 ppm peanut protein) and dark chocolate (2 and 20 ppm peanut protein) to discern sensitivity and quantitative accuracy.

Results: Optimization steps improved method sensitivity between 5 and 100-fold, depending on peptide and matrix (e.g., detection reduced from 50 to 0.5 ppm peanut protein for NLP in dark chocolate). In the method evaluation, robust detections of the lowest level of peanut-incurred food matrices were observed (1.24 and 2 ppm peanut protein in cookie and dark chocolate, respectively). Recovery of peanut protein ranged from 42.43-456.81% in cookie and 44.40-288.02% in dark chocolate. The method detected and quantified peanut contamination at levels sufficiently sensitive to protect the peanut-allergic population.

Significance: The developed method effectively detects low levels of peanut in processed food matrices, which is an identified shortcoming of other analytical methods. Orthogonal and confirmatory use of this method would increase confidence in analytical results, increase safety for peanut-allergic consumers, and aid in risk-based decisions in the industry.

T7-04 Accelerating the Detection of Bacteria in Food Using Artificial Intelligence and Optical Imaging

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Introduction: Early detection of microbial contamination in food products is critically important for consumer safety and outbreak prevention. However, conventional culture-based methods require the isolation of bacterial macro-colonies for biochemical or genetic characterization, which could take a few days and is labor-intensive.

Purpose: This study aimed to develop a rapid, user-friendly, and affordable method for detecting multiple bacterial species in food products.

Methods: The detection is based on artificial intelligence (AI)-enabled analysis of morphological differences among bacterial microcolonies. Bacteria were cultivated for 3 h on a non-selective growth medium to form microcolonies. Phase contrast microscopy was used to obtain the images of bacterial microcolonies, generating 2,520 images from eight common foodborne bacteria such as *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes*. Bacterial species classification was performed using the real-time object detection algorithm called You Only Look Once version 4 (YOLOv4). Both pure bacterial culture and food samples were tested. This method was validated by standard plating assay.

Results: YOLOv4 was able to discriminate eight common foodborne bacterial species with an average precision of 94%. This approach also enabled rapid quantification of bacterial concentrations over three orders of magnitude with the R^2 of 0.995. For romaine lettuce spiked with *E. coli* (10 - 10^3 CFU/g), the trained YOLOv4 detector had a false-negative rate of less than 10% (n=12).

Significance: This AI-assisted detection not only achieves high accuracy in bacterial classification but also provides the potential for automated bacterial detection, reducing labor workloads in food industries, environmental monitoring, and clinical settings.

Technical

T7-05 The Devolvement of Polymer-Based Sensors for Detecting Antibiotics in Food

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Introduction: Antimicrobial resistance (AMR) has recently been established as a leading cause of death worldwide. A key factor in combating AMR is monitoring and limiting antibiotic levels in food. Polymer-based sensors possess unique properties, which give them great potential for the sensitive and specific detection of antibiotics in food.

Purpose: The study developed a novel polymer-based sensor to identify an antibiotic in food products.

Methods: Molecularly imprinted polymers (MIPs), which act as synthetic antibody mimics, were synthesized for the detection of the antibiotic tetracycline. The sensor was validated with tetracycline-spiked buffered solutions using a novel dual detection system of thermal and fluorescent analysis. Spiked egg samples were also analyzed using thermal detection to demonstrate proof-of-application in real food samples.

Results: The thermal response to tetracycline was compared for the developed MIP and a reference non-imprinted polymer. The MIP exhibited a 430 times greater thermal response to tetracycline compared to the non-imprinted polymer and its limit of detection was 0.1 mM, which is below the legal maximum residual levels. Furthermore, a measurable thermal response was observed in tetracycline-spiked egg samples. This demonstrates initial proof-of-application for in-field tests using food samples. Tetracycline has many derivatives with similar structures and therefore it is likely that MIPs can not only detect tetracycline individually, but other derivatives also.

Significance: The developed MIP can detect an antibiotic in buffered solutions and real food samples with high sensitivity. This highlights that MIPs possess a higher level of matrix versatility compared to gold standard antibody testing. Furthermore, unlike antibodies, MIPs are low-cost and compatible with portable detection. This creates the possibility for widespread on-site screening using MIP-based sensors, which will facilitate the determination/quantification of contamination sources without requiring a lab environment. The technology is also highly versatile and has been adapted for the detection of norovirus and SARS-CoV-2.

T7-06 Enrichment-Free Detection and Speciation of *Listeria monocytogenes*, *Listeria* spp. and *Salmonella* spp., Based on a Multiplexed Isothermal RNA Amplification, Coupled to DNA Microarray Hybridization

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Introduction: *Listeria monocytogenes, Listeria spp* and *Salmonella spp* contamination have emerged as a major concern for Food Safety, but the present detection methods are either culture based and slow, or PCR based and fast, but less sensitive than culture methods and with only modest multiplex capability. Thus, a fast, sensitive, multiplexed and enrichment-free test is needed for the Food Processing environment.

Purpose: To develop an enrichment-free, isothermal nucleic acid assay that can detect *Listeria monocytogenes*, *Listeria* spp and *Salmonella spp,* with multiple specificity controls, in parallel as a single test, in less than 4hrs from a single surface swab or sponge.

Methods: We have invented a ***N***ucleic ***A***cid ***S***equence ***B***ased ***A***mplification (NASBA) microarray technology for rapid detection of up to 100 RNA targets simultaneously at the SNP level. In the present deployment of the technology for Food Safety, "Envirox-f Gen2", a set of multiplex NASBA-microarray amplification primers and microarray hybridization probes were designed for 16S and 23S rRNA targets, as well as a mRNA target for *L. monocytogenes.* This multiplex assay has been tested using 23 cultured ATCC and USDA bacterial standards in cell suspension and contrived into EZ Reach sponges.

Results: This assay correctly identified multiple validated *L. monocytogenes*, *Listeria* spp. *and Salmonella* spp. training samples, without culture enrichment, from 23/23 isolates at a cLOD of 10^0 CFU/ml which is equivalent to that obtained via BAM-standard 36 h plate culture.

Significance: This assay allows simultaneous detection of *L. monocytogenes*, *Listeria* spp. *and Salmonella* spp. without culture enrichment at a time scale suitable for same-shift testing. These data demonstrate feasibility of the technology as a lab-based test, with a 4 h workflow, and for future deployment as an autonomous system for "Point of Swab" analysis. The technology can also be extended to the analysis of other panels of bacterial, viral, and fungal pathogens.

T7-07 Genomic Detection of *Salmonella* in Chicken Meat Samples Using an End-to-End Nano-Biosensor Platform

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Introduction: *Salmonella* is a foodborne bacterial pathogen responsible for thousands of hospitalizations and hundreds of deaths in the United States alone each year. It can be contracted by consuming contaminated meat products. Conventional detection methods are laborious and time-consuming, requiring overnight culturing in various growth media.

Purpose: To develop a simple, rapid, and cost-effective end-to-end nanoparticle-biosensor platform to detect *Salmonella* in chicken cuts and ground chicken.

Methods: The samples analyzed in this study are store-bought cuts of chicken and ground chicken. Twenty-five grams of the meat samples were first inoculated with *Salmonella* to simulate contamination. Using glycan-coated magnetic nanoparticles, the bacteria were extracted and concentrated from the sample matrix. Bacterial DNA was extracted and used directly without amplification in a gold nanoparticle-based plasmonic DNA nano-biosensor detecting the invA gene of *Salmonella*. The biosensor signal can be discerned using either the naked eye or a smartphone application.

Results: The magnetic nanoparticles could concentrate the cells from the meat samples, as observed in the growth media. *Salmonella* was successfully detected in contaminated samples with a detection limit as low as 1 ng of dsDNA/μL. The biosensor signal can be discerned within 10 minutes, and the whole analysis can be accomplished within 4 hours from sample preparation to detection results.

Significance: The nano-biosensor platform significantly reduces the waiting time for the analysis results, which can lead to safer meat products and increased foodborne disease prevention. It can be potentially used as a time-efficient and cost-effective way to monitor the presence of *Salmonella* at various points in meat processing plants and the supply chain.

T7-08 Magnetic Nanoparticles, a Potential Biosensor to Aid in the Rapid Molecular Detection of *Salmonella* Typhimurium at Sub-Infectious Dose Levels

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❖ ***Developing Scientist Entrant***

Introduction: Salmonellosis caused by *Salmonella* species is of global concern due to the ubiquitous and zoonotic nature of the pathogen. It is among the top-priority pathogens in Food Safety and responsible for many illnesses and death in the United States. Therefore, rapid detection of *Salmonella*, more importantly at lower infectivity doses, will be an important step in disease prevention. As an evolving technology, using magnetic nanoparticles (MNPs) in medicine, agriculture, and other fields has significantly advanced various research. Nevertheless, the application of this technology to aid in concentrating and extracting foodborne bacteria has not been extensively investigated.

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Purpose: This study uses glycan-coated magnetic nanoparticles (MNPs) to extract and concentrate *Salmonella* Typhimurium from broth cultures. The non-interference of MNPs with Real-time Polymerase Chain reaction (RT-PCR) detection of the pathogen is investigated.

Methods: Briefly, a 0.5 McFarland turbidity of bacteria suspension was prepared, and 1:3 serial dilutions of four concentrations were made. The approximate bacteria concentration from the first to the last dilution (1:84) was 1.5×10^5 to 1.9×10^2 cells/µl. These were used for magnetic capture, plating, and RT-PCR experiments.

Results: There was an efficient extraction of *Salmonella* Typhimurium by MNPs from both reference and wild strains at all levels of bacteria concentrations, even to the fourth dilution corresponding to 5.7×10^2 CFU/µl, where the MNPs comparatively captured more bacteria (*p-value 0.007).* Furthermore, the presence of MNPs in RT-PCR reactions did not inhibit amplification but aided in the efficient molecular detection of sub-infectious dose level *Salmonella* directly used as part of the reaction mixture.

Significance: The findings of this study expand our knowledge on the rapid detection of pathogens even at very low infectivity levels considering that the infectious dose of non-typhoidal *Salmonella* is approximately 10^3 bacilli.

T7-09 Nanoparticles for Multiplex Genomic Detection of Carbapenem-Resistant *E. coli* in Food Samples

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial-resistant bacteria are a major growing concern worldwide. Recently, cases of carbapenem-resistant bacteria have globally increased at an alarming rate, which is mainly a result of rapid dissemination of carbapenemase through horizontal gene transfer (HGT). Several studies reported that carbapenemase-producing (CP) bacteria were not only found in clinical samples but also in municipal wastewater, drinking water, surface and ground water, agricultural environments, food animals, and retail food products. Transmission of CP bacteria also alarms food safety, contributing their spread worldwide due to the global food trade, which poses a risk to human health. The surveillance has been extended to tracking CP pathogens (e.g. *E. coli* and *Salmonella*) in food animals and meat products since 2016 in the U.S. and the Europe.

Purpose: This study presents a rapid and simple platform for detection of carbapenem-resistant *E. coli* from food samples (chicken and lettuce).

Methods: This study used glycan-coated magnetic nanoparticles (MNP) for rapid extraction of bacterial cells and dextrin-capped gold nanoparticles (dGNP) for rapid genomic detection. After DNA extraction of MNP-bacteria mixture, a colorimetric biosensor utilizing the stability of dGNP (red color) was developed with specific probe, *uidA* for *E. coli* and *blaKPC* for carbapenem-resistance, allowing the detection of unamplified DNA in 30 min. The colorimetric detection was quantified with absorbance spectra measurements.

Results: Successful detection of susceptible and resistant *E.coli* extracted from food at initial contamination approximate 10^3 CFU/ml was achieved. Further, the detection was achieved in the presence of natural microbiota and food microparticles. This platform was also able to differentiate susceptible *E. coli* from carbapenem-resistant E. *coli*. Detailed results and discussion will be presented.

Significance: Successful detection of carbapenem-resistant *E. coli* in the food samples was achieved in < 7 h with magnetic extraction, a short enrichment step, and visual detection while the traditional methods take 18 to 72 h.

T7-10 Simultaneous Quantitative and Qualitative Analysis of PFAS in Food Using the ZenoTOF 7600 System

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Introduction: Food testing is critical for understanding the dietary exposure of PFAS in humans. The variability in the type of PFAS and their concentrations in food necessitates an analytical method with a wide dynamic range and complementary compound identification.

Purpose: A newly launched accurate mass spectrometer was used to develop a method that can simultaneously quantitate at low ng/ml levels and perform high-resolution confirmation of PFAS during targeted and non-targeted analysis of food matrices.

Methods: Using the ZenoTOF 7600 system, an MRM method was developed to analyze 16 PFAS with a calibration range of 0.01 to 25 ng/ml. In the same method, the MS/MS fragments were used to confirm the identity of the target PFAS by library matching. Different food samples were processed using the FDA method for PFAS analysis in food and screened by non-targeted acquisition using SWATH.

Results: The sensitivity enhancement from the mass spectrometer enabled the quantitation of 16 PFAS compounds with LOQs ranging from 0.01 to 0.05 ng/mL and provided high-resolution confirmation of their presence and even some matrix interferences based on MS/MS library matching. Accurate masses were used to distinguish known interferences such as taurine-based cholic acids from PFOS and even unknown interferences from PFBA and PFPeA as mitigation against reporting false positives in certain foods. Non-targeted screening using SWATH acquisition confirmed the presence of PFHpS, PFOS, PFNA, PFDA, and FOSA in a contaminated meat sample.

Significance: A highly sensitive LC-MS/MS method capable of quantifying PFAS with LOQs as low as 0.01 ng/mL and providing high-resolution identification was developed. The latter is important for PFBA and PFPeA since both are typically difficult to confirm due to having a single MRM transition. Improvement in MS/MS sensitivity resulted in the production of high-quality MS/MS spectra for more confident identification of PFAS via library matching during targeted and non-targeted screening workflows.

T8-01 Influence of Biofilm Architecture on Sanitizer Tolerance of *Listeria monocytogenes* from Artisanal Cheese Environments

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❖ ***Developing Scientist Entrant***

Introduction: Contamination of ready-to-eat foods by *Listeria monocytogenes* chiefly occurs post-processing via cross-contamination with food environmental strains which persist through forming sanitizer-tolerant biofilms.

Purpose: This study explored biofilm structural differences that may explain sanitizer tolerance in mature (10 day) biofilms of *L. monocytogenes* from artisanal cheesemaking environments.

Methods: Six strains of *L. monocytogenes* isolated from Vermont artisan cheese operations from 2006-2008 were cultured in 1X and 1/20X Brain heart infusion (BHI) broth adjusted to OD_{600} 0.05-0.10. Each culture was inoculated into petri dishes containing 6 stainless steel coupons and incubated statically at 23°C for 10 days with nutrient replacement every 48 hours. Four coupons were then rinsed 3X with PBS and treated with a Quaternary Ammonium Compound (QAC) or Sodium Hypochlorite (SH) sanitizer at 0, 50, 100 and 200ppm, and enumerated by spot plating on BHI agar. The remaining two coupons were rinsed, treated with 0ppm or 200ppm QAC or SH and prepared for Scanning Electron Microscope (SEM) imaging using standard methods.

Results: Under nutrient poor conditions (1/20X BHI), *L. monocytogenes* isolates appeared as infrequent cell aggregates interspersed with single cells wedged inside surface micro fissures. Nutrient rich samples (1X BHI) showed dense, consistent single-cell coverage with more frequent 3D aggregates. Isolates 9 and 19, isolated from a floor squeegee and cheese fill hoops, respectively, had especially dense coverage in nutrient rich samples, with substantial 3D development within surface micro fissures on the coupon. In nutrient poor conditions, these isolates also showed greater exopolysaccharide development than the other four isolates. Both QAC and SH sanitizers showed incomplete biofilm destruction regardless of nutrition, condition, with unharmed single cells and cell aggregates scattered across the coupons.

Significance: *L. monocytogenes* displays substantial variation in biofilm architecture, but even the more primitive biofilm architectures impart significant sanitizer tolerance.

Technical

T8-02 Longitudinal Survey of Food Safety Hazards in a Commercial Recirculating Aquaponics System

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❖ ***Developing Scientist Entrant***

Introduction: Aquaponics is a sustainable agricultural method that minimizes the use of resources by using by-products from fish for produce production. Few studies have investigated the prevalence and distribution of foodborne pathogens in commercial systems.
Purpose: This longitudinal study aimed to identify several foodborne pathogens, their potential biotic and abiotic indicators, and their distribution throughout a commercial, recirculating aquaponic system.
Methods: Over 2 years (0-671 d), a microbial evaluation was performed on a controlled environment aquaponics farm containing Nile tilapia (*Oreochromis niloticus*) and hydroponically grown lettuce (*Lactuca* sativa). Samples (*n*=1,044) were collected bimonthly from each system, including lettuce, roots, fingerlings (7-126 d old), fish feces (>126 d old), water, and sponge samples from the tank interior. Most probable number of generic *Escherichia coli* were determined using IDEXX Colilert Quanti-Tray. Enumeration and enrichment were used to detect Shiga-toxigenic *E. coli* (STEC), *Salmonella enterica*, *Listeria monocytogenes*, *Aeromonas* spp., *Aeromonas hydrophilia*, and *Pseudomonas aeruginosa*. Isolates were confirmed using real-time PCR.
Results: Generic *E. coli*, STEC, *L. monocytogenes,* and *S. enterica* were not detected from any samples. *P. aeruginosa* was isolated from water (7/351; 1.99%), sponge (3/351; 0.85%), fish feces (2/108; 1.85%), and lettuce (2/99; 2.02%) samples. *Aeromonas* spp. was quantified in all sample types. Sponge, water, and root samples collected within the first 6 months had less *Aeromonas* than between 8-22 months (2.82±0.12 vs. 4.06±0.60 log CFU/cm^2, 2.39±0.45 vs. 2.98±0.58 log CFU/ml, and 4.06±0.36 vs. 5.39±0.82 log CFU/g, respectively; P<0.05). *A. hydrophila* was isolated from all sample types (544/1,044; 52.1%). The age of the system affected *A. hydrophila* presence in water (X^2=23.234, P<0.001) and sponge samples (X^2=21.352, P<0.001).
Significance: *Aeromonas* and *A. hydrophila* were significantly affected by the system's age; after six months of production, contamination by this pathogen in an aquaponic system may pose a risk to fish health and lettuce-associated foodborne illness in humans.

T8-03 Predicting Disinfectant Resistance in *Listeria monocytogenes U*sing Whole Genome Sequencing and Machine Learning

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❖ ***Developing Scientist Entrant***

Introduction: Even though there are many studies that focus on the prediction of antimicrobial resistance from bacterial genomic data, a similar prediction of resistance to disinfectants remains very poorly studied.
Purpose: The objectives of this study are to i) obtain a high performing predictor of resistance to disinfectants (Benzalkonium chloride and Peracetic acid) and ii) explore prediction of minimum inhibitory concentration (MIC) values.
Methods: Within this study, 1650 *Listeria monocytogenes* whole genome sequencing (WGS) samples and their corresponding MIC values were used to train different Machine Learning models. We constructed the pangenome using Prokka and Roary and called single nucleotide polymorphism variants using Samtools and GATK. Absence and presence tables of these different genomic features were subsequently used for the ML prediction. We explored a range of standard ML models, as well as a symbolic regression model (QLattice) and a group-testing based model (INGOT-DR). All ML algorithms were designed both as a classification task (i.e. resistant, susceptible) and a regression task (MIC values).
Results: We were able to train high performing ML learning models for classification and regression. For the classification task the results show that a linear logistic regression model with l1 regularization performs best achieving a balanced accuracy of 0.91. For the more difficult regression task we found that random forest regressors (RFR) outperform the others with r2-scores up to 0.68. Additionally, the test set results for RFR show 287/330 predictions that are within one-dilution difference of the measured MIC. We will also report important features used by the algorithms to make predictions which could indicate possible resistance determinants.
Significance: The results of this study enable a better understanding of *L*. monocytogenes resistance towards different disinfectants used in the food industry. Our predictive models could further support case specific adaptation of cleaning procedures in case of *L. monocytogenes* contamination among production sites.

T8-04 Phytochemicals Modulate *Listeria monocytogenes* Proteome Critical for Infection in Humans

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* (LM) is a major foodborne pathogen that leads to life-threatening illness in humans. The current listeriosis treatment includes antibiotic therapy. However, there are reports of antibiotic resistance development in LM. This situation warrants the development of novel strategies for controlling LM infection in humans.
Purpose: This study investigated the effect of Generally Recognized as Safe status phytochemicals namely Trans-cinnamaldehyde (TC), Eugenol (EG), and carvacrol (CAR) on the proteome profile of LM, especially the expression of proteins critical for infection in humans.
Methods: Sub-inhibitory concentrations (SICs) of TC, EG, and CAR against LM ATCC-19115 were determined by growth curve assay at 37°C. For proteome profiling, LM ATCC-19115 was cultured either in the presence (treatments) or absence (control) of SICs of phytochemicals for 12 h at 37°C 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 on Scaffold-5 at P<0.05.
Results: The SIC of TC, EG, and CAR was 0.01%, 0.04%, and 0.008%, respectively. Approximately 1350 proteins were identified of which ~ 97 proteins were down-regulated and ~ 281 proteins were upregulated by the phytochemicals (P<0.05). All phytochemicals down-regulated critical virulence proteins contributing to cell invasion (InlB), intra-cellular survival and spread (PlcA, ActA), toxin production (Hly), cold and oxidative stress tolerance (CspD, CspLA, RsbT), protein synthesis (InfA), catalytic activity (PyrC, PbpA, PyrF), energy metabolism (RmpF2), fatty acid biosynthesis (AcpP) and translation (RplQ, RplL) (P<0.05). Corresponding genes related to above mentioned proteins (*inlA, plcA, actA, hly*) were also reduced in expression (P<0.05). The expression of a few proteins involved in catalytic activity and cellular metabolism (Gap, ldh1, FusA, GroES, Map, GatB, PflA) were upregulated by phytochemicals (P<0.05).
Significance: These results delineate the potential mechanism of action of TC, EG, and CAR against LM and provide a basis for future studies investigating the anti-listerial efficacy of the aforementioned compounds in an animal model.

T8-05 Simulation Evaluation of Power of Sampling Plans to Detect Cronobacter in Powdered Infant Formula Production

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❖ ***Developing Scientist Entrant***

Introduction: *Cronobacter* is a risk in Powdered Infant Formula (PIF) products that is hard to detect due to localized, low prevalence, and low-level con-

Technical

tamination.

Purpose: We adapt a sampling simulation tool for PIF sampling and benchmark industry-relevant sampling plans for detecting different *Cronobacter* contamination scenarios.

Methods: The simulation was first validated by reproducing published probabilities of detecting 0-10% prevalence contamination events with systematic grab samples (n=10, 25, 75) and systematic increment samples (n=750). Then published contamination profiles of a recalled PIF batch [42% prevalence, -1.779±0.675 log(CFU/g)] and a reference PIF batch [1% prevalence, -2.44±0.8 log(CFU/g)] were used to benchmark representative sampling plans [(n=30,m=10g), (n=30,m=25g), (n=60,m=25g), (n=180,m=25g)]. The recalled batch profile was used to compare 300g total mass sampling plans with different number of grabs (n=1, 3, 5, 10, 30, 60, 100, 22000). To compare the different number of clusters (n=1, 5, 10, 50, 100) with 10% total prevalence and -1.779 ± 0.675 log(CFU/g) contamination level, a representative sampling plan (n=30,m=10g) was simulated. For every simulation, Simple Random Sampling, Stratified Random Sampling, and Systematic Sampling were iterated 10,000 times.

Results: Simulating the different number of grabs with 300g total mass showed that taking more than 30 grabs had a <1% median probability to accept the recalled batch. Benchmarking representative sampling plans with recalled batch profile showed that simulated representative sampling plans would reject the contaminated lot (<1% median probability of acceptance) but it would rarely reject the reference batch even with the most stringent sampling plan (n=180,m=25g) (>50% median probability of acceptance). When the number of clusters increases, the difference between sampling strategies decreases, as the same contamination becomes better mixed.

Significance: The simulation will help industry stakeholders design science-based sampling plans by entering parameters describing their hazard scenarios and sampling plans of interest.

T8-06 Incidence of Multiserovar *Salmonella* Populations in Postharvest Meat and Poultry Products

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❖ ***Developing Scientist Entrant***

Introduction: Despite significant reduction of *Salmonella* incidence during processing, meat and poultry products remain a considerable source of foodborne salmonellosis. Conventional *Salmonella* monitoring often relies on the identification of a single serovar from a sample, which causes some serovars to remain undetected.

Purpose: This study was designed to determine the incidence of multiserovar *Salmonella* populations in meat and poultry following antimicrobial processing interventions.

Methods: Deep serotyping with CRISPR-SeroSeq is a next-generation sequencing approach that quantifies the relative abundance of serovars within a mixed population. We applied CRISPR-SeroSeq to analyze 93 surveillance samples collected at slaughter facilities across the United States (pork: n=40; chicken: n=39; beef: n=9; turkey: n=5). Concurrently, up to two colonies were isolated and serotyped using molecular methods. A subset of isolates (~20%) were selected for confirmatory testing using traditional antibody agglutination methods.

Results: CRISPR-SeroSeq identified 33 serovars, including 8/10 most frequently found by the CDC. There was an average of 1.4 serovars per sample, with a maximum of five serovars detected within a single sample, and 25.8% (24/93) of samples contained multiple serovars. Alternatively, conventional isolation and serotyping methods found 29 serovars (average of 1.1 serovars per sample) and 7.53% (7/93) of samples contained multiple serovars. Serovar profiles differed between commodity types, with Eko, Kentucky I, Montevideo I, and Hadar most often found in pork (25.0%; 10/40), chicken (43.6%; 17/39), beef (44.4%; 4/9), and turkey (40.0%; 2/5) samples, respectively. In 89/93 instances, identified serovars matched between serotyping and CRISPR-SeroSeq.

Significance: Population analyses by CRISPR-SeroSeq revealed a quarter of post-harvest, post-intervention samples contain multiple serovars, demonstrating the complexity of *Salmonella* populations and the limitations of conventional methods. In six instances, CRISPR-SeroSeq additionally identified *Salmonella* serovars Enteritidis, Typhimurium, or Newport, that were undetected by traditional culture methods. This study highlights the importance of comprehensive pathogen surveillance monitoring in food production systems.

T8-07 Quantification of Foodborne Viruses and Its Correlation with Somatic Coliphages in Leafy Greens Vegetables

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne viral outbreaks linked to fresh produce have been increasing, and detection may be difficult to achieve. The use of microbial indicators potentially can indirectly estimate the load of viral pathogens.

Purpose: To evaluate the correlation level between foodborne viruses and somatic coliphages for use as a viral indicator.

Methods: Three hundred twenty samples of vegetables were collected,160 of each lettuce and parsley. From these, 40 composite samples (4 samples per composite) for lettuce and parsley were subjected to both, a TGBE buffer elution and PEG 8000/ NaCl precipitation. To estimate the recuperation efficiency of this method, samples were artificially spiked with Murine norovirus (10^6 to 10^0 total gc). Somatic coliphages were quantified by the EPA 1602 method, and hepatitis A virus (HAV), human norovirus (NoV GI, GII and GIV) and Rotavirus A (RoV) by RT-qPCR. Correlation analysis of the quantification of foodborne viruses and somatic coliphages were determined by R studio.

Results: Our PEG 8000/NaCl concentration-based protocol shows an efficiency of recovery of 22.66±22.86% and 14.51±14.84% in lettuce and parsley respectively. A total of 9/40 (22.5%) in lettuce and 8/40 (20%) in parsley samples tested positive for RoV and none for Norovirus or hepatitis A. Quantification of RoV range from 2.53×10^1 to 2.94×10^4 total genomic copies in lettuce samples and 2.19 to 2.07×10^4 in parsley. The range of somatic coliphages quantification were 1 to 10×10^3 PFU/100ml in both, lettuce, and parsley. Spearman´s correlation between the concentration of RoV and the somatic coliphages indicated an r2=-0. 4885243 ($P<0.05$) in lettuce, while on parsley r2=-0.1448956 ($P<0.05$).

Significance: These findings suggest a potential use of somatic coliphages as viral indicators of the absence of RoV in fresh produce, although a larger study involving more, and different samples would validate this correlation.

T8-08 Prevalence and Risk Factors for Self-Reported Diarrheal Illness in Communities of Three Regions of Ethiopia

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❖ ***Developing Scientist Entrant***

Introduction: Low- and middle-income countries (LMICs) are disproportionately affected by foodborne disease (FBD), although data on the health

impacts of FBD are limited. Understanding the prevalence, potential exposures, and risk factors for diarrheal disease will allow for quantification of the burden of FBD, inform efforts to prevent FBD, and reduce FBD mortality rates.

Purpose: The objective of this study is to quantify self-reported diarrheal disease and identify potential risk factors in three regions of Ethiopia.

Methods: A cross-sectional survey was conducted from October 2021 – October 2022 in three regions of Ethiopia. Randomly selected households were interviewed about household characteristics, diarrheal illnesses, healthcare behaviors, food consumption, and environmental exposures. Descriptive statistics were used to summarize household characteristics. Logistic regression was used to estimate prevalence and identify risk factors for self-reported diarrheal illness.

Results: Participants from 2,436 households were interviewed in Addis Ababa (n=812), Harar (n=812), and Gondar (n=812), Ethiopia. The prevalence of household self-reported diarrheal illness was 10.34%. Odds of self-reported diarrheal illness were 3.518 (95% CI: 2.649, 4.671) times higher in males compared to females; 1.143 (95% CI: 1.079, 1.212) times higher for each additional individual in the household; 1.717 (95% CI: 1.225, 2.407) times higher in households with goats compared to households without goats; 2.139 (95% CI: 1.286, 3.560) times higher in households using untreated water compared to those using treated water; and 2.842 (95% CI: 1.661, 4.862) times more likely in households with no latrine facility (open fields) compared to households using a piped sewer system or septic tank.

Significance: Results showed sex, household size, toilet type, water treatment, and household ownership of goats are significant risk factors for diarrheal illness and warrant further study to estimate their role in the overall burden of FBD in Ethiopia.

T8-09 Rapid and Non-Destructive Prediction of Pork Microbial Quality Using Volatolome-Based Artificial Neural Networks

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❖ ***Developing Scientist Entrant***

Introduction: The generation of volatile organic compounds (VOCs) and the associated off-odors are amongst the typical microbial spoilage phenomena for pork. Selected-ion flow-tube mass spectrometry (SIFT-MS) is a novel analytical technique effective for quantifying these compounds at trace levels in real-time.

Purpose: This study aims to predict the microbial quality of pork, by developing a multidisciplinary methodology that combines volatolome analysis and data-driven modelling.

Methods: Fresh pork loin was stored at 4 °C under high-O_2 (air, 70/0/30, 70/30/0 – %O_2/CO_2/N_2) and low-O_2 (5/30/65, 0/30/70, 0/0/100 – %O_2/CO_2/N_2) atmospheres. Each packaging condition was tested twice using two different initial pork meat batches. In total, 225 storage samples (6 conditions by 2 batches by 6-7 measurement days by 3 daily replicates) were analyzed by classical microbiological plating and SIFT-MS. Back-propagation neural networks (BP-NN) were then established based on the quality of each individual sample, with partial least squares regression (PLS-R) as a comparison. Logarithmic and standardized concentrations of VOCs were used as model inputs, and logarithmic bacterial counts as outputs. Two high-O_2 and two low-O_2 conditions were set for training and validation by turns, and the remaining conditions left out for testing.

Results: Acetoin and 3-methyl-1-butanol were greatly accumulated in high-O_2 spoilage, while ethanol, 3-methylbutanal, H_2S, and methyl mercaptan were characteristic for low-O_2 atmospheres. The volatolome-based BP-NN outperformed the conventional PLS-R model and had satisfactory power in predicting total colony counts, especially for low-O_2 conditions (accuracy factor: 1.044-1.068, root mean square error: 0.370-0.492). Using BP-NN, 73 to 80% of samples stored under low-O_2 atmospheres can be predicted within ± 0.5 log CFU/g of estimation residuals for total colony counts.

Significance: The implementation of fast non-invasive/non-destructive techniques such as SIFT-MS is expected to accelerate future quality indication in meat supply chains, especially in tandem with machine learning methods.

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T8-10 A Machine Learning Approach to Identifying *Salmonella S*tress Response Genes in Isolates from Poultry Processing

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* is exposed to stressors including low and high temperatures, disinfectants, and low nutrients at various stages of the poultry processing cycle. The development of stress responses could induce cross-protection to other stressors or cause variations in *Salmonella* pathogenicity. For instance, the NusA protein which is synthesized under cold temperature conditions has been found to influence the expression of the transcriptional activator of *Salmonella* pathogenicity island-1, *hilA*. The identification of significant genes across specific product-pathway conditions could improve microbial risk models.

Purpose: The purpose of this study was to utilize machine learning algorithms to identify *Salmonella* stress response genes in isolates from poultry processing.

Methods: Genome assemblies of *S. enterica* isolates (n=189) from chicken sources were downloaded from the National Center for Biotechnology Information (NCBI) Pathogen Detection database and annotated on the eggNOG-mapper (v2) platform. The annotated data was explored for class imbalance and the presence of near-zero variance features. Six algorithms were employed to model the data in a supervised machine learning approach using R (v 4.2.2). Subsequently, the best-performing model was used to rank genes that were significant to the model classification.

Results: All models showed a classification performance, based on the area under the receiver operating characteristic (AUROC), above 0.85. Logit boost algorithm showed the highest AUROC of 0.9 and also achieved superior confusion matrix statistics (Kappa=0.81; sensitivity=0.89; specificity=0.92). A number of genes associated with *Salmonella* heat and cold stress response, transcriptional regulation, transport mechanisms, and cellular metabolism were identified.

Significance: Machine learning algorithms can accurately classify *Salmonella* whole genome sequences (WGS) and identify relevant genes that can be further used in developing WGS-based quantitative microbial risk assessment models.

T8-11 Comparison of Three Air Sampling Methods for the Quantification of *Salmonella*, Shiga-Toxigenic *Escherichia coli* (STEC), Coliforms, and Generic *E. coli* Bioaerosols from Cattle and Poultry Farms

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Introduction: Recent fresh produce outbreaks potentially associated with bioaerosol contamination from animal operations in adjacent land highlighted

the need for research data to understand the associated risk.

Purpose: To evaluate three sampling methods for quantifying target bacterial bioaerosols from animal operations.

Methods: A confined cattle and a poultry farm located in Southeastern, US were visited six times each, respectively. Air was collected for 10 min using: 2-stage Andersen impactor with and without mineral oil overlay and impingement samplers. Sampling devices were run concurrently at the animal operation border at 0.1, 1, and 2 m heights (n=30). Andersen samplers were loaded with CHROMagar™ *Salmonella* and STEC, or Brilliance™ coliforms/*E. coli*. The impingement sampler contained buffered peptone water (20 ml) which was vacuum filtered through a 0.45 µm filter and placed onto the respective media. Plates were incubated at 37 °C for 48 h. PCR confirmation followed targeting *ttr* for *Salmonella* and stx_1, stx_2, and *eaeA* genes for STEC.

Results: No significant differences were found among methods to quantify coliforms and *E. coli* bioaerosols. Coliforms and *E. coli* bioaerosol concentrations were significantly higher in poultry than in cattle farms ($P<0.05$). *Salmonella* and STEC bioaerosols were not detected by any of the methods assessed (Limit of detection: 0.55 log CFU/m^3). Coliforms and *E. coli* bioaerosols were similarly distributed at both stages in the Andersen samplers (stage 1:>7 µm; stage 2: 0.65–7 µm particle size). Mineral oil did not enhance the recovery of bioaerosols in this instance, which had been shown for recovery in other situations. Sampling day did not have a significant effect on the recovery of coliforms and *E. coli* bioaerosols from poultry farms ($P>0.05$).

Significance: These data will help determine effective bioaerosol sampling techniques which can be used for the detection of bacterial foodborne pathogens and indicator organisms for future research.

T8-12 Low to Zero Concentrations of Airborne Bacterial Pathogens and Indicator *E. coli* in Proximity to Beef Cattle Feedlots in Imperial Valley, California

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❖ ***Developing Scientist Entrant***

Introduction: California Leafy Green Products Handler Marketing Agreement (LGMA) established food safety metrics for producing leafy greens, with guidance recommendations of 400 feet, 1200 feet, and 1-mile distances between production fields and either a composting facility utilizing animal products, or a feedlot containing >1000 or >80,000 head of cattle, respectively.

Purpose: The purpose of this study was to evaluate this effect of distance from cattle feedlots on the occurrence of airborne *E. coli* O157, non-O157 Shiga-toxin–producing *E. coli* (STEC), *Salmonella*, and indicator *E. coli* and to identify environmental risk factors associated with these airborne bacteria.

Methods: Each sample comprised 6000 liters of air with TSB-enriched air filters qPCR-screened for pathogens and *E. coli*; suspect positive colonies were further qPCR-confirmed. A separate air sample was collected for direct enumeration of *E. coli*. Local meteorological data was collected *in situ* and from nearby weather stations, along with events of dust-generating activity in line-sight distance.

Results: No bacterial pathogens were qPCR-confirmed for the 300 samples totaling 1.8 million L of processed air. Indicator E. coli was detected in 16.7% (50/300) of samples, with positives found at all distance categories. Logistic regression on all 300 air samples found significantly higher odds of *E. coli* for samples taken in close proximity compared to >2000-ft distance from a feedlot. Environmental factors associated with *E. coli* detection included wind speed, wind direction, relative humidity, sampling hour of day and month, and the presence of dust-generating activities (cattle activity, plowing fields).

Significance: Lack of bacterial pathogen detection within these sampled distances suggests airborne deposition from nearby feedlots may not be a significant mechanism of leafy green bacterial pathogen contamination; detection of very low concentrations of indicator *E. coli* as a function of distance, wind speed, and direction provides data to inform future revisions of produce safety guidance documents such as the California LGMA.

T9-01 Resuscitation of Viable-but-Nonculturable *Campylobacter jejuni* in Embryonated Chicken Eggs

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter jejuni* is one of the leading causes of gastrointestinal diseases worldwide. It can enter a viable-but-nonculturable (VBNC) state to survive under stresses. VBNC cells demonstrate pathogenic potentials due to their ability to resuscitate at favorable conditions, posing a serious health risk. However, resuscitation of VBNC *C. jejuni* has not been well understood.

Purpose: This study aimed to induce VBNC *C. jejuni* at low temperature (4°C) and investigate its resuscitation in embryonated chicken eggs.

Methods: Four *C. jejuni* strains were separately stored in Mueller Hinton (MH) broth at 4°C. The number of VBNC population was monitored using quantitative polymerase chain reaction coupled with propidium monoazide (PMA-qPCR) combined with the plating assay. VBNC cells were aseptically inoculated into 9-day-old embryonated chicken eggs and then incubated at 37°C for up to 3 days. Egg contents were collected and homogenized, followed by testing the culturability of *C. jejuni* on MH blood agar.

Results: *C. jejuni* strains were induced into VBNC state (with final concentration of ~10^6 CFU/mL) at 4°C after 28 to 49 days depending on the strains. All 4 strains of VBNC *C. jejuni* were able to resuscitate after incubation in embryonated chicken eggs with resuscitation rates ranging from 65% to 90.5%. The identity of the resuscitated bacterial cells were verified using 16S rRNA sequencing. Embryonated eggs from 3 different chicken breeds were confirmed to allow the resuscitation. Inoculation numbers of at least 10^4 CFU of VBNC cells were required to enable the resuscitation. The resuscitation of VBNC cells were observed starting from 1-day post-inoculation.

Significance: C. jejuni can enter VBNC state at 4°C and resuscitate in embryonated eggs. The knowledge from this study can facilitate a better understanding of the health risks associated with VBNC *C. jejuni* and provide insights to ensure safe food processing and storage conditions.

T9-02 Effectiveness of Meat Hygiene and Safety Training Intervention for Mitigating Risk of Coliform Contamination Levels of Raw Beef in Selected Butcher Shops in Addis Ababa, Ethiopia

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne disease (FBD) is a major public health concern in developing countries where inadequate food safety measures, lack of infrastructure, and poor food safety and hygiene practices, contribute to the spread of diseases caused by contaminated food, particularly FBD attributed to foods of animal origin.

Purpose: The objective of this study was to evaluate the effectiveness of a meat safety and hygiene training intervention at beef butchery shops in Addis Ababa, Ethiopia.

Methods: Butcheries that receive beef carcasses from municipal abattoirs were enrolled in the study between November 2021 thru May 2022. Representatives from the butcheries provided a one-day training in concepts in basic meat safety and hygiene. Carcass swabs were collected from the flank, lateral thorax, and brisket pre- and post-training. Swabs were taken both first thing in the morning when carcasses first arrived from the abattoir, and 2-8 hours after the initial swab. Swabs were analyzed for total coliform counts using standardized methods. Descriptive statistics and linear regression were used to determine the mean total coliform counts pre- and post-training and the impact of the training intervention on risk of contamination.

Results: A total of 60 butcheries were enrolled in the study and 240 carcass swabs were collected and analyzed. Pre-training morning and afternoon mean total coliform counts were 3.91 (SD=0.876) and 4.3 (SD=1.02) log CFU/cm^2, respectively. Post-training morning and afternoon mean total coliform

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counts were 4.44 (SD=1.22) and 4.47 (SD=1.20) log CFU/cm^2, respectively. Morning and afternoon total coliform counts were significantly correlated (R^2= 0.74 ;95% CI: 0.647–0.812), indicating the positive relationship. After adjusting for this correlation, the meat safety and hygiene training intervention was marginally associated (p-value=0.08) with a decrease in contamination.

Significance: These findings show that the implementation of a combination of pre- and post-slaughter measures will reduce the risk of contamination of raw beef in butcher shops.

T9-03 The Multiple Dilemmas of Cooked Meat Products Suppliers: Offering Safe Products, Saving Cost and Following Customers Trends… Fermentation Can Help!

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Introduction: cooked meat is regarded as a high-risk food for *Listeria monocytogenes* (*Lm*) contamination. Despite good hygiene and manufacturing practices after cooking there are still some sensitive steps where the products are re-manipulated and can be contaminated. Which strategy can be applied to help suppress the growth of *Lm*?

Purpose: The purpose was to evaluate if the addition of a food culture (*Latilactobacillus curvatus*) can be used to help suppress to growth of *Lm* in hot dog sausages.

Methods: 100% pork hot dog sausages were sprayed with food culture (concentration between 6.3 and 7.0 log CFU/g) and vacuum packed. 20 Challenge tests (following ISO 20976-1:2019 or EURL Lm technical guidance document) using 20 different Lm strains were initiated considering a shelf life up to 60 days at refrigerated and abuse temperatures. Depending on the study, post pasteurized or non-post pasteurized sausages containing 108 ppm of nitrite or nitrite-free were used as a control.

Results: the validity of challenge tests was first assessed based on the Lm standard deviation at D0 (<0.3 Log CFU/g). Growth potential (GP) were compared based on average concentration at D0 and at each sampling time during the shelf life. The spray-on application of *Latilactobacillus curvatus* helps suppress the growth of *Lm*. No significant growth was observed on no nitrite nor on non post-pasteurized sausages containing culture (GP < 0.5 Log CFU/g while the GP was > 5 log CFU/g in the batches without culture). The drop in pH was faster with culture, but the final pH difference between the control and the batches with culture was between 0 and 0.4 pH.

Significance: The addition of properly selected food culture help suppress the growth of *Lm* and at the same time help suppliers improving margin (via removing post-pasteurization) and quality of their product while answering consumers request for clean label meat products

T9-04 Compliance with Food Safety Standards by Beef Vendors at Butcheries in Kamuli District, Uganda

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Introduction: Butcheries are the leading retailers of beef in Uganda but their level of compliance with food safety standards is unknown.

Purpose: This study aimed to determine if beef vendors in Kamuli district followed the US 736:2019 standard for Hygienic requirements for butcheries.

Methods: Based on the standard, a validated survey questionnaire and observation checklist on sanitation, hygiene, meat storage, demographics, and butchery operations were used to collect data from 60 butcheries in Kamuli, Uganda. Descriptive statistics including percentages and proportions were used for analysis. Observed and self-reported beef vendors' practices were compared to the requirements in the food safety standard to identify any gaps that needed to be addressed.

Results: Most beef vendors complied with the inspection, storage, and some sanitation and hygiene requirements of the standards, but violations occurred within the transportation and construction requirements. From the self-reported survey, 96.7% (n=58) of vendors sold inspected beef, 83.3% (n=50) of butchery facilities were inspected at least once a month and all vendors stored meat for less than 36 hours with most storing the meat by hanging inside the butchery (67.9%; n=36). Additionally, 76.7% (n=46) of beef vendors reported washing handling tools with water and soap, 50% (n=30) packaged beef in woven polypropylene bags, 60% (n=36) transported beef to butcheries using motorcycles, and 96.7% (n=58) cleaned butcheries every day. During the observational assessment, all beef vendors had short hair, short fingernails and did not wear jewelry, 90% (n=54) of butchery walls were dirty, and flies were present at 80% (n=48) of facilities. Butcheries were constructed with wooden walls (71.7%, n=43), and their floors were either wooden or bare ground (65%, n=39). Only 15% (n=9) of beef vendors wore protective clothing when handling meat.

Significance: Food safety interventions with beef vendors need to focus on the safe transportation of meat, construction of butchery facilities, and the use of personal hygiene practices.

T9-05 Results of a Multi-Year Inter-Laboratory Proficiency Testing Program for Zearalenone in Corn

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Introduction: Proficiency testing programs are important to measure the reliability of results from HPLC/MS determination of mycotoxins in ground corn.

Purpose: This research provides results from a double-blind study of accuracy, precision, and reproducibility for zearalenone quantitated in paired ground wheat and corn samples determined by HPLC/MS over several years.

Methods: Double-blind ground corn samples with known zearalenone levels were analyzed by each laboratory. Neogen's sample preparation used five-gram samples extracted in 70% methanol/water + 125ng of ^{13}C zearalenone. Filtered and centrifuged samples were diluted in PBS and passed over immunoaffinity columns prior to HPLC/MS analysis. Samples from the same lot were analyzed once per year for five years. Statistical analysis including ANOVA was completed using Minitab 18 (Minitab, LLC).

Results: The inter-laboratory mean for four laboratories was 94.2±16.4 ppb zearalenone (CV=17%, n=12) for a ground corn check sample containing nominal 90.5 ppb zearalenone; 218.4 ± 22.4 ppb (CV = 10.3%, n=12) for a 273.0 ppb sample and 1105.4 ± 149.6 (CV = 13.5%, n=12) for a 1004.6 ppb sample. Over five years of testing, six laboratories participated. For the 90.5 ppb sample, standard deviations (SD) were <9 ppb for results from samples of the same lot for all laboratories except one lab that had a statistical outlying result and another lab that had 16.7ppb SD for three samples in one year. For the 273.0 ppb sample, SDs were <32 ppb for all labs except results from one lab that had statistical outliers from previous year results. For the 1004.6 ppb sample, standard deviations were <115 ppb for all labs.

Significance: The comparison of multi-year results provided information about the quality of the ground corn check samples and long-term laboratory proficiency.

T9-06 Rapid Quantitative Analysis of Fraudulent Olive Oils Using Recurrent Neural Networks and Raman Spectroscopy

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Introduction: The 2020-2021 Canadian Food Inspection Agency's (CFIA) annual report on food fraud shows expensive edible oils are more likely to be substituted or diluted than other food categories. To identify the adulteration of edible oils, fast, low-cost, and universally applicable non-targeted screening methods are needed. This study investigates the application of recurrent neural networks (RNNs) and Raman spectroscopy for determining the oil types and their corresponding percentages in fraudulent olive oils. RNNs are commonly used for temporal problems, such as speech recognition. Here we consider a Raman spectrum as sequential data and carry out regression using RNNs.

Purpose: To determine the oil types and their percentages in fraudulent olive oils.

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Methods: Extra virgin olive oils were mixed with seven other edible oils, including sunflower, soy, peanut, corn, canola, grapeseed, and avocado oils. All oils were purchased from local grocery stores, and only 100% pure oils were used. Samples were placed in a 96-well plates and covered with coverslips. Raman spectra were collected using a Raman microscope with a 532 nm laser and a 60x objective lens. The exposure time was 10 sec. A total of 25 Raman spectra were collected for each oil sample, followed by data training using RNNs.

Results: RNNs and Raman spectroscopy allowed us to obtain a continuous regression for the adulterant percentage in olive oil over the range of 1 to 99% (v/v). Our approach identifies both the adulterant oil types and their percentages with an error of ~2% (root mean square), which is within the AOAC standard of 5% for Non-Targeted Testing of Ingredients for Food Authenticity/Fraud Evaluation of Extra Virgin Olive Oil.

Significance: Our study provides a rapid quantitative analysis of adulterants in olive oils. The method has the potential to be a fast, low-cost, and user-friendly solution for the food oil industry.

T9-07 Rapid Pomegranate Juice Authentication Using a Simple Sample-to-Answer Hybrid Paper/Polymer-Based Lab-on-a-Chip Device

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Introduction: As a super fruit, pomegranate and its juice have attracted increased consumer demands during the past decades. Given the high production cost and market price, adulteration of pomegranate juice is highly likely to occur.

Purpose: This study aimed to develop a rapid and simple sample-to-answer analytical device for the authentication of pomegranate juice and detection of cheaper fruit juices potentially being added into pomegranate juice, such as apple and grape.

Methods: Pure pomegranate juice and juice mixtures were prepared in the lab from whole fruits. Loop-mediated isothermal amplification (LAMP) reactions were designed for pomegranate, apple and grape, individually. Harvesting the advantages of paper materials and polymer materials, a hybrid paper/polymer sample-to-answer microfluidic "lab-on-a-chip (LOD)" device was designed to integrate DNA extraction, LAMP reaction, and LAMP results visualization onto one single device.

Results: This LAMP-based authentication method achieved highly sensitive (*i.e.*, 10 pg for pomegranate DNA and 100 pg for grape and apple DNA) and specific detection of pomegranate, apple, and grape DNA present in fresh fruit juice. Allowing a simultaneous detection of three species of fruit, the LAMP-LOC device developed in this study was able to detect 2 µL of fresh pomegranate juice and 5 µL of fresh apple and grape juice. Using a homemade portable heating device, the overall analysis could be completed in ~1 h without the need of any laboratory specific facilities. The cost for each authentication test was estimated to be ~4 USD and the reusable homemade portable heating device was ~15 USD.

Significance: This LAMP-based simple sample-to-answer hybrid paper/polymer-based LOC device has high potential to be adopted by government laboratories and the food industry to rapidly and routinely authenticate pomegranate juice even in a resource-limited environment.

T9-08 Characterization and Selection of Lactic Acid Bacteria for the Development of a Direct-Fed Microbial in Food Animals

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❖ ***Developing Scientist Entrant***

Introduction: Shigatoxin-producing *Escherichia coli* (STEC) and *Salmonella* are important foodborne pathogens, commensal to the animal GI-tract. Reduction of these pathogens in food-animals could prevent their introduction into the food-chain. Direct-fed-microbials (DFM) are live microbial cultures exhibiting antagonism against specific microorganisms in the GI-tract. Lactic acid bacteria (LAB) may be utilized as effective DFM, with careful selection and screening.

Purpose: Characterization and selection of LAB for development of DFM in food animals.

Methods: Viability of 250 LAB strains was determined after extended frozen storage (-80°C), by determining growth under optimal conditions (24-48h @) 37/42°C ; anaerobic incubation). Those showing excellent or very-good growth were evaluated for tolerance to acid (pH 2, 4, 5, 7) and bile (0, 0.1, 0.3, 0.5%) at 0, 1, 3, and 6 h; inhibition against pathogens ($1.0x10^4$ CFU/ml) – STEC (O157, O111, O103, O45, O26, O121, O145) and *Salmonella enterica* Typhimurium, using agar-spot-test; and resistance against common antibiotics (ampicillin, clindamycin, erythromycin, gentamycin, streptomycin, tetracycline, vancomycin), through disk-diffusion assay. Selected LAB were also evaluated for their biofilm-forming ability (48 h at 37/42°C) on polystyrene surface.

Results: Of the 250 LAB strains, 65 were tested further, as described above. Among those, 15% exhibited excellent (>15 mm), 32% very-good (>10 mm), and 29% good (>5 mm) inhibition against tested pathogens. Thirteen percent showed excellent, 35% very-good, and 39% good tolerance to acidic conditions, while 29% exhibited excellent, 16% very-good, and 52% good tolerance to bile. All the LAB strains were capable of forming biofilms, with 33% forming strong, 50% moderate, and 17% weak biofilms. All tested LAB were resistant to streptomycin and gentamicin, followed by vancomycin (67%), tetracycline (50%), ampicillin (42%), and clindamycin (25%). None of the LAB were resistant to erythromycin.

Significance: Selected LAB strains could be used for DFM development based on their survival under stress conditions and inhibition against foodborne pathogens.

T9-09 From Bacteriophage-Supplemented Feed to *Salmonella*-Free Poultry

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Introduction: *Salmonella*-contaminated poultry feed is a major source of flock infections, but the use of phage additives in feed is a safe and effective method of breaking this transmission route.

Purpose: The purpose of this study was to prove the effectiveness of BAFASAL™, a GRAS bacteriophage preparation active against *Salmonella,* in protecting poultry feed from contamination.

Methods: Commercial complete poultry feed has been sprayed-on with bacteriophage cocktail (1×10^5 PFU/g) and experimentally contaminated with environmental *Salmonella* Enteritidis isolated from poultry farm (1×10^3 CFU/g). The experiment was performed twice with two different strains: SE65 and SE12. Positive control samples and phage treated feed contaminated with *Salmonella* were incubated in triplicates at 30°C. *Salmonella* load was determined after 6 h and 24 h. The *Salmonella* load in the evaluated samples was recalculated into the % of pathogen reduction by bacteriophages compared to control samples. Statistical analysis was performed by one-way ANOVA followed by Tukey post hoc test. The quality of feed was confirmed by the phage stability storage tests at 4°C and 25°C.

Results: In the tested feed samples with phages, the load of *Salmonella* SE65 was significantly reduced to 0.72% (1.03×10^8 CFU/g vs 1.43×10^{10} CFU/g) and *Salmonella* SE12 was significantly reduced to 0.65% (2.18×10^7 CFU/g vs 3.38×10^9 CFU/g) after 24 h ($P \leq 0.01$). Thus it can be concluded that bacteriophages can reduce number of *Salmonella* in experimentally contaminated feed by more than 99%, when applied at a dose of 10^5 PFU/g. The stability storage tests confirmed 99% activity of bacteriophages at 4°C and 87% activity at 25°C after one month.

Significance: The obtained results indicate that bacteriophage cocktail is an effective tool for providing safe feed for poultry and in a wider context can protect animals from feed originating infections.

T9-10 Pet Owner Perceptions and Practices Regarding Raw Meat-Based Pet Diets in the UK and Slovenia

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❖ ***Developing Scientist Entrant***

Introduction: Raw meat-based diets do not undergo any processing to eliminate pathogens which may pose risk to human health. In the absence of safe food-handling practices, proliferation of such pathogens may cause a severe foodborne illness to pet owners and other members of the household. It is vital to understand current perceptions and practices of pet owners providing raw pet diets in different countries.

Purpose: This study compared the findings from Slovenian and UK surveys regarding pet owner perceptions and practices when providing raw meat-based diets to pets.

Methods: Findings from two surveys conducted in the UK (n=174) and Slovenia (n=382) were compared and narratively interpreted with consideration for possible regional and cultural differences. Survey questions included: pet owner motivation to provide raw meat; confidence in safe raw meat-based food preparation; confidence in cleaning and sanitizing; awareness of risks to human health. Practices included: defrosting, handwashing, cleaning pet bowl and rinsing raw meat.

Results: Study determined that UK pet owners were less aware (36%) of potential lethal outcomes of a foodborne illness, compared to Slovenian pet owners (78%). In both studies majority of pet owners have shown high confidence in the ability to prepare raw meat-based food safely (UK-90%; Slovenia-94%). Despite this, malpractices were reported, such as unsafe defrosting (UK-57%; Slovenia-42%), inconsistent hand hygiene after pet food preparation (UK-6%; Slovenia-10%) and rinsing raw meat (UK-27%; Slovenia-67%).

Significance: The findings from UK and Slovenia indicate similar trends in perceptions and practices among raw meat-based feeding pet owners; one exception was the practice of rinsing raw meat, which could be country-specific and requires further investigation. Study determined that pet owners may be overly optimistic about the competence to prepare raw meat-based food safely. These findings could inform the design of future interventions and comprehensive instructions, raising awareness and encouraging safe raw meat-based pet food preparation practices.

T9-11 What are the Factors Affecting the Efficacy of a Natural Anti-*Salmonella* Solution in Fat and Animal Meal?

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Introduction: Fat and animal meal are key raw materials in the pet food industry but highly sensitive to *Salmonella* post-cooking contamination. Preservatives are therefore needed to ensure microbial safety, but several factors can influence their inhibitory efficacy.

Purpose: This study aims to measure the impact of some factors such as impurities, type of fat, moisture, temperature, and dosage on the anti-*Salmonella* efficacy of a natural preservative.

Methods: Antimicrobial efficacy was measured with the logarithmic reduction of *Salmonella* over time under changing conditions and expressed as kinetics or area under curve. An experimental design was used for some factors and the physiological stress of *Salmonella* cells was examined with the selective medium plating technique.

Results: The level of impurities up to 2% in poultry fat didn't significantly impact the efficacy of a natural preservative (*p-value*=0.48). However, the performance was moderately altered (*p-value*=0.02) for fat with moisture values up to 3%, well above recommended limits (2.5%). The physiological stress of *Salmonella* cells shows, in the presence of a preservative, a maximal level for the highest values of moisture and impurities, interestingly opposed to the results without preservative. In addition, the preservative efficacy seems only slightly impacted (*p-value*=0.04) by the time spent in fat. It's notable that, for the same level of quality, beef fat shows the greatest impact on the efficacy of the preservative (*p-value*<0.05). Finally, the temperature plays an important role in antimicrobial efficacy, either directly as for fat, or indirectly as for meal, by potentiating the effect of the antimicrobial.

Significance: Several factors seem to impact the efficacy of preservatives, in particular moisture level and the type of fat and of course temperature, that is a key point. Finally, to ensure an optimal microbial safety, it is crucial to take care of the raw materials quality in addition to the use of a natural preservative.

Technical

T9-12 Development of a Radio-Frequency Technology for the Decontamination of *Salmonella* from Timothy Hay

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Introduction: Radiofrequency (RF) heating utilizes high-frequency electromagnetic waves in the RF range to generate heat in a targeted material or substance.

Purpose: The objectives of this research were to: 1) Investigate the efficacy of RF heating on the decontamination of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 in Timothy Hay, 2) Evaluate the suitability of *E. faecium* as a surrogate of *Salmonella* in Timothy hay during RF treatment, and 3) Assess the physicochemical changes after RF treatment on the Timothy hay on vitamins, amino acids, fatty acids, and trace minerals.

Methods: A pilot-scale parallel-plate RF heating system (6 kW, 27.12 MHz) was used to conduct this study. Timothy Hay was procured from a pet food manufacturing plant in Lincoln, Nebraska, at an initial MC of 7% to 9% (wet basis). Timothy hay samples (150 g) were inoculated with either a cocktail containing five serotypes of *Salmonella* or a broth of *Enterococcus faecium,* then incubated at 37◦C for 24±2 h. Timothy hay samples were exposed to RF energy for 165, 175, 185, and 195 s. This resulted in 12 samples (4 heating durations x 3 replicates). A one-way fixed effects ANOVA and Tukey's HSD test were performed to determine significant differences between samples.

Results: After RF treatments, Salmonella loads were reduced to 5.80±0.24, 4.00±0.27, 1.42±2.01 log CFU/g and below the detection level after 195 s. At 165 and 175 s of RF treatments, the *E. faecium* loads were reduced to 7.50±0.14 and 6.39±0.31 log CFU/g and below the detection level at 185 and 195 s. There were no statistically significant changes in the Iron, Vitamin A, or Amino Acid responses because of increasing RF treatment duration. However, sodium levels increased, and potassium and calcium levels decreased in response to the increasing RF treatment durations.

Significance: The study demonstrated a non-chemical approach to decontaminating *Salmonella* and its surrogate, *Enterococcus faecium,* from low-moisture foods such as pet foods.

T10-01 Development of a Novel Multiplex Probe-Based, Real-Time PCR Assay for Simultaneous Detection of EHP and WSSV Infections in Shrimps

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Introduction: Shrimp farming contributes around 53% of global seafood production. Losses due to *Enterocytozoon hepatopenaei* (EHP) and *white spot syndrome virus* (WSSV) infection are amongst the most significant in shrimp aquaculture. Thus, early diagnosis with rapid and reliable detection of these viruses is essential for quick surveillance and control.

Purpose: To develop a valid method for simultaneous detection of EHP and WSSV infections in shrimps.

Methods: EHP and WSSV specific gene targets were chosen for developing the multiplex assay. Target specific primers and probes were designed and

evaluated on synthetic DNA at different concentrations and temperature conditions. Assay sensitivity, specificity, repeatability and robustness were performed according to the FDA guidelines. Assay validation was carried out on EHP and WSSV positive and negative shrimp samples. The DNA from shrimp samples was extracted using HiPurA® Shrimp DNA Purification kits (manual and magnetic). All magnetic extraction and PCR assays were performed on HiMedia's InstaNX®Mag16/InstaNX®Mag32 nucleic acid extractor and InstaQ 96 plus RT-PCR machines, respectively.

Results: HiMedia's Hi-PCR® EHP and WSSV detection multiplex probe PCR assay was developed with high specificity, sensitivity and robustness. The sensitivity attained was 2.82 copies/μL and 141 copies/μL for EHP and WSSV, respectively. A 100% specificity was obtained for both targets when evaluated with the other reported Shrimp infecting pathogens. Also, no variation in results were observed with change of person and machine indicated the robustness of the assays. Overall, the developed assay was found to be specific, sensitive, robust, reliable and expeditious with analysis times less than 70 minutes (less than 3 hrs including extraction).

Significance: HiMedia's Hi-PCR® EHP and WSSV detection multiplex probe PCR assay coupled with extraction offers complete solution for automated and simultaneous detection of EHP and WSSV in shrimps. It will aid as a reliable molecular tool for early detection of shrimp infections, thereby prevention of disease outbreaks and economic losses.

T10-02 Evaluation and Modeling the Shelf Life of Shrimps Under the Frozen Temperatures

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Introduction: The online on-demand food delivery service is experiencing explosive growth. Some seafood products like shrimps require frozen temperatures during transport and storage to maintain the quality of this seafood. However, temperature fluctuation frequently occurs during transport or storage due to, among others, loading/unloading activities.

Purpose: The purpose of this study was to evaluate the shelf-life of shrimps (*Litopenaeus vannamei*) under frozen temperature regimes.

Methods: Shrimp samples were purchased from a farmer in Yilan, Taiwan. Samples were cleaned and stored in various frozen temperature levels (-5, -10, -15, and -20°C). The shelf-life was determined by evaluating the changes in chemical and microbiological properties as well as the sensory attributes. The changes were then analyzed using Arrhenius kinetic model.

Results: The TVBN values (Total volatile basic nitrogen) of the shrimp stored at -5 and -10°C exceeded the spoilage level (25 mg/100g) after 1.5 and 7 months of storage, respectively. Conversely, the TVBN value of this seafood stored at -15 and -20°C did not reach the spoilage level even after 12 months of storage. Furthermore, this study observed that the total viable count of all the shrimp samples kept under -5, -10, -15, and -20°C did not reach the spoilage level (6 log CFU/g) after 1.5, 7, 12, and 12 months of storage, respectively. Based on sensory evaluation, the shrimp samples stored at -5, -10, -15, and -20°C were rejected by the participants after 1.5, 5, 11, and 12 months of storage, respectively, due to melanosis problems.

Significance: Based on chemical and microbial properties, keeping the shrimp under -20°C could preserve the shelf-life of shrimp for more than 12 months. However, it was rejected by the participants after 12 months of storage due to the sensory changes. Allowing the temperature fluctuation higher than -20°C would result in a shorter shelf-life of this seafood.

T10-03 Use of Digital PCR (dPCR) as a Complimentary Method for Detection of *Cyclospora cayetanensis*

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Introduction: Digital PCR (dPCR) is a technology used for quantification of microorganisms and differs from traditional quantitative PCR (qPCR). In dPCR DNA samples are separated into thousands of micro-partitions, allowing for attributing amplification of target(s) to a specific molecule.

Purpose: Evaluation of the dPCR technology for *Cyclospora cayetanensis* research via detection of a mitochondrial gene target and compare the advantages and limitations, data generated, and quantified results of dPCR to qPCR.

Methods: A library of different *C. cayetanensis* DNA samples was processed using the standard method (BAM Chapter 19c) and qPCR data was generated (Ct values from 22.58 to 36.35). Samples were run in QIAcuity Digital PCR and data output were compared qualitatively.

Results: The dPCR readings per partitions are presented in copies/µl and the threshold can be independently defined per sample. qPCR data were reported as cycle threshold data (Ct) or copies/µl when compared to standard curves. qPCR had one defined threshold for all samples (0.08) while dPCR allowed individual threshold setting per samples to differentiate background from amplified fluorescence. Lowest and highest threshold were 61.46 and 64.01, respectively. All samples previously detected by qPCR were detected by dPCR. The protocol that provided best results contained primers and probe in the final reaction at the concentration of 0.8µM each.

Significance: dPCR can provide absolute quantification without the need of standard curves. This method is sensitive according to our tests, however more samples from different matrices (food and water matrices) need to be tested to evaluate the performance of the method when inhibitors are present. Absolute quantification is important since different laboratories from government agencies or state health department may need to compare results with standardized methods. Our findings suggest that dPCR may contribute as a complementary method for the detection of foodborne parasites in combination with current standard protocols.

T10-04 Transfer of SARS-CoV-2 Surrogate Bacteriophage Phi6 from Tomatoes to Gloves to Cucumbers and Its Persistence on Discarded Gloves

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Introduction: The preventive measures employed to control SARS-CoV-2 dissemination includes the use of plastic gloves during fresh-produce shopping. Little is known about the transfer of SARS-CoV-2 from gloves to fruit and its persistence on discarded gloves.

Purpose: This study assesses the transfer of the SARS-CoV-2-surrogate bacteriophage Phi6 from tomato to high density polyethylene (HDPE) plastic gloves to cucumber, and its persistence on used gloves at room temperature.

Methods: Phi6 was propagated using *Pseudomonas syringae* pathovar *phaseolicola* as host on Luria-Bertani agar (LB) plates using the double agar overlay method. LB plates were incubated overnight at 25°C and viral titer enumerated for all the sampling points indicated below. Phi6 was spot inoculated on the surface of sanitized tomatoes (6 log PFU/ml) and dried for 1 h. The forefinger of a gloved hand (~4 cm² contact area) was kept in contact without pressure with the inoculated surface for 2, 5, 10 or 30s. The same gloved forefinger was then pressed for 2, 5, 10 or 30 s onto a sanitized cucumber. Bacteriophage phi 6 persistence on HDPE gloves after 30s of contact with tomatoes was also measured at 25°C over 9 days.

Results: Phi6 transfer from tomato to gloves increased with increased contact time. The highest transfer of Phi6 from tomato to glove was observed after 30 s (4.8 log PFU/sample; 81%), and after 30 s from gloves to cucumber (2.2 log PFU/g; 46%). Phi6 counts decreased by approximately 2.8 and 3.4 log PFU/glove after 3 and 6 days of storage, respectively. Viral particles on gloves were below detection limit (1.5 PFU/sample) after 9 days.

Significance: Phi6 can be transfer from HDPE gloves to fresh fruit suggesting SARS-CoV-2 will as well. Phi6 transferred from fruit to plastic gloves can persist at 25°C. These results will be useful for managing SARS-CoV-2 risk in fresh produce and on gloves.

T10-05 Evaluation of a New Automated Viral RNA Extraction Platform on at-Risk Food Matrices

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❖ ***Developing Scientist Entrant***

Introduction: Produce and mollusks are often associated with foodborne viral outbreaks, such as human noroviruses (NoV) and hepatitis A virus (HAV). As these viruses are the leading cause of foodborne illnesses worldwide, their detection in foods is critical.

Purpose: Herein, we optimized and validated an automated extraction platform for viruses in several food matrices. The optimized method was compared to a semi-automated method using the Boom methodology for nucleic acid extraction as suggested by the reference method ISO 15216-1:2017.

Methods: Fresh or frozen raspberries, frozen blackberries (25 g), romaine lettuce (25 g) and oyster digestive glands (2 g) were spiked with NoV GII.4 and GI.7 and HAV at either 10^2, 10^3 or 10^4 genome copies in deionized water/sample. The ISO method was used for the elution, recovery, and purification of all samples. Viral RNA was extracted using the semi-automated or the automated platform. A column purification was also added after the extraction on the automated platform to further remove RT-qPCR inhibitors. All experiments were replicated three times.

Results: While optimizing on frozen raspberries, the elution of HAV RNA (10^3 copies/sample) from the silica beads at the end of the extraction allowed a detection at lower Cq for the automated platform (10 min, 80°C) than the semi-automated platform (5 min, 60°C). The addition of a column purification after the extraction also allowed a better detection (P=0.0404 and P=0.0082 respectively, two-way ANOVA). For NoV GII.4, the effect of the optimized extraction was significant (P=0.0183). For the comparison between the two platform, results were analyzed in a qualitative detection (presence/absence of signal), the two methods showed similar results for the five food matrices studied.

Significance: These results show that the automated extraction platform can be performed easily by users without constant attention while obtaining equivalent and even superior results, thus showing its potential for routine analysis through food processing and surveillance.

T10-06 Trends in Reported Illness Due to Poultry- and Non-Poultry Associated *Salmonella* Serotypes

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Introduction: Despite the reported reduction of *Salmonella* prevalence in poultry products, the reported overall incidence of *Salmonella* infection in the United States did not decline between 1996–2019; however, temporal patterns vary among *Salmonella* serotypes.

Purpose: The objective of the study is to analyze trends in reported illness due to poultry- and non-poultry associated *Salmonella* serotypes.

Methods: The analysis considers the available FoodNet *Salmonella* serotype human annual incidence data for 1996-2019. Serotypes associated with poultry are identified based on a recent report by the National Advisory Committee on Microbiological Criteria for Food (NACMCF) and Food Safety Inspection Service (FSIS) sampling of chicken parts during 2015-2021. Statistical analyses are divided into two parts. The first analysis is a generalized additive model (penalized B-spline regression) of *Salmonella* serotype incidence trends that produces 95% confidence bands around the estimated curves. The second is a compositional data analysis that evaluates temporal changes in the proportion of culture-confirmed *Salmonella* incidence due to identified serotypes, with trend tested using the non-parametric Mann-Kendall procedure.

Results: The analyses demonstrate significant declining trends in the incidence and proportion of poultry-associated *Salmonella* serotypes (P<0.05) and significant increasing trends in the *Salmonella* serotypes not associated with poultry (P<0.05).

Significance: While the declining trend in the incidence of illness due to poultry associated *Salmonella* serotypes is consistent with the reported reduction of *Salmonella* prevalence in poultry products, it does not necessarily follow that the decline can be attributed to the decreased prevalence in poultry products. However, the increasing trend in the incidence of non-poultry associated *Salmonella* serotypes cannot be attributed to poultry products.

T10-07 Diarrhea Illness Management and Associated Costs in Healthcare Facilities in Ethiopia

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❖ ***Developing Scientist Entrant***

Introduction: Healthcare facility-level data on diagnostic, treatment practices and patient costs for diarrheal illness can help characterize the extent and nature of Food Borne Diseases (FBDs) and the capacity of countries to manage food safety risks.

Purpose: To characterize health-facility management practices and costs of diarrheal illness in Ethiopia.

Methods: A cross-sectional survey of healthcare facilities in Addis Ababa, Harar, and Gondar, Ethiopia was conducted from October 2021 to September 2022. A list of health facilities, including private and public, was obtained from regional health offices in the study sites and 179 facilities were selected using stratified random sampling. One administrator and up to three workers were interviewed in each facility to assess diagnostic and treatment practices as well as costs associated with diarrheal illnesses.

Results: Facilities in Addis Ababa (100%) and Gondar (80%) served urban/peri urban dwellers while those in Harar served mostly rural (77%) communities. Laboratories were available in 60% of facilities; less than half had pediatric units. The median number of diarrhea patients seen per worker per week was 4 (IQR 1 - 10) and stool specimens were obtained from half of these patients. Samples were primarily tested for protozoa (36%) and bacteria (5%). Oral fluids and antibiotics were prescribed, on average, to 60% and 55% of diarrhea patients, respectively. The median cost of one healthcare provider visit for diarrhea varied from 0.09 to 0.71 USD while laboratory testing of a single stool specimen costed 0.62 USD.

Significance: Diagnostics-based management of diarrhea is rare in healthcare facilities in Ethiopia. Bacterial testing of stool samples is rare, but prescription of antibiotics to treat diarrhea is not uncommon and these results can guide efforts to understand how FBD diarrhea management could be improved in the country.

T10-08 Creation of a Novel Foodborne Illness Disease Surveillance Approach Combining Wastewater-Based Epidemiology and Social Media Semantic Filtering

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Introduction: Traditional foodborne illness outbreak identification relies on passive surveillance and is subject to delays in case reporting, resulting in prolonged retail of contaminated food and increased human exposure. Interdisciplinary novel modern approaches to active disease surveillance are needed to accelerate outbreak identification and provide investigators earlier warning. Wastewater based epidemiology (WBE) has been demonstrated to provide signals of human disease prior to identification of cases. Online discussions and posting in social media have been shown to occur when individuals are ill.

Purpose: To optimize an approach model to validate a novel active surveillance system by combining two continuously monitored community disease

surveillance approaches: pathogen identification through WBE and social media semantic filtering (SMSF) of online discussions.

Methods: Over a 8 month period, wastewater was sampled weekly on a University campus, and samples were evaluated using real-time polymerase chain reaction (RT-PCR) and metagenomics for a variety of human pathogens. Human case data was obtained from the University and local Public Health Unit. When WBE identified a signal which was associated with human cases, a retrospective semantic filtering of social media related to a variety of symptoms was conducted to evaluate whether online discussions geofenced to the ultra-local areas where clusters were identified.

Results: WBE identified 4 illness clusters or sporadic cases during the sample period in specific dormitory residences. Retroactive SMSF showed an increase of symptomatic discussions and key words prior to case reporting in a similar timeframe as WBE. These clusters were subsequently confirmed through traditional public health approaches (clinical and symptomatic evaluation).

Significance: Monitoring conducted in a geographically localized early warning system permitted the early detection of illness outbreaks in a single community. Preliminary work demonstrates that there is value in combining WBE with SMSF and this approach provides a model for the early identification of foodborne pathogens in other locations.

T10-09 Prevalence and Risk Factor Identification of Foodborne Illness Associated Pathogens in Laboratory Confirmed Cases of Enteric Infection in Ethiopia

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❖ ***Developing Scientist Entrant***

Introduction: Enteric diarrheal disease is a major contributor to morbidity and mortality worldwide; however, in Ethiopia, there is little information on the incidence of clinically confirmed enteric infections. Understanding the prevalence of enteric infections is necessary for determining the burden of foodborne disease in Ethiopia and identifying risk factors associated with illness.

Purpose: The objective of this study is to estimate the prevalence of laboratory-confirmed parasitic and bacterial infection and identify risk factors associated with illness.

Methods: Laboratory results and associated patient metadata for stool samples tested at the clinical microbiology and parasitology laboratories in three hospitals in Ethiopia from 2018 through 2020 were collected and digitized. Descriptive statistics were used to summarize laboratory test results. Logistic regression was used to identify factors associated with positive test results.

Results: Of 27,592 fecal samples collected, 29.65%, 5.00%, and 65.35% were tested, respectively, for parasites, bacterial pathogens, and both parasites and bacteria. The proportion of fecal samples positive for at least one microbial pathogen was 0.1336 (CI: 0.1296, 0.1376). Adults were 1.463 (CI: 1.350, 1.586) times more likely to have a fecal sample positive for a bacterial or parasitic pathogen compared to children. Additionally, region was significantly associated with a positive sample; samples from Gondar (OR: 4.307 [CI: 3.815, 4.861]) and Harar (OR: 4.470 [CI: 3.759, 5.315]) were more likely to test positive compared to those from Addis Ababa. Compared to the dry season, samples collected during light rain (OR: 1.137 [CI: 1.039, 1.243]) and long rain seasons (OR: 1.136 [CI: 1.043, 1.239]) were more likely to be positive.

Significance: Region, age, and season were significantly associated with having a fecal sample test positive for an enteric pathogen. Further investigation of the role of these factors in enteric diarrheal disease will contribute to better understanding of the burden of diarrheal disease in Ethiopia.

T10-10 Investigating the Food Sources of Extended-Spectrum B-Lactamase-Producing *E. coli* Causing Community-Acquired Urinary Tract Infections in Bangladesh: A Molecular Epidemiological Study

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Introduction: Multidrug resistant (MDR) *Escherichia coli* causing urinary tract infections (UTI) are presumed to have originated in poultry and transmitted to humans via the food chain. There is insufficient evidence from epidemiological studies to recognize that food is a source of MDR uropathogenic *E. coli* (UPEC).

Purpose: Identify foodborne sources of MDR UPEC by sampling both community-acquired UTI (CA-UTI) patients and high-risk food sources contemporaneously in Dhaka, Bangladesh and analyze the isolates for phylogenetic relatedness.

Methods: During 2017–2018, 2297 food samples including raw poultry meat (n=970), fresh-produce (n=504), raw fish (n=334), raw eggs (n=327) and raw beef (n=162) samples collected from 14 wet-markets in Dhaka were tested for *E. coli* and ESBL-producing *E. coli* (ESBL-Ec). During this time, 112 ESBL-Ec strains isolated from 394 CA-UTI patients living in the same areas were included for comparative analysis. Phylogroups of food and clinical ESBL-Ec were identified, and isolates belonged to the same phylogroups were analyzed by whole genome sequencing (WGS).

Results: About 87% (n=1993) food samples were *E. coli* positive, of which 30% (n=600) were ESBL-Ec. Poultry meat was predominantly positive for ESBL-Ec (43%, n=421) followed by raw beef (36%, n=58), raw fish (24%, n=74), eggs (12%, n=22) and fresh-produce (6%, n=25). Majority of clinical ESBL-Ec (67%) belonged to phylogroup B2 but none of the food isolates belonged to this phylogroup. Phylogroups A, C, D, E and F were common in both sources comprising 33% of clinical and 84% food ESBL-Ec isolates. WGS analysis revealed that only 3 clinical ESBL-Ec matched with poultry isolates by their phylogroups (D, F, F), multi-locus sequence types (ST38, ST457, ST648) and serotypes (O1:H18, O11:H25, O102:H6).

Significance: Except for three isolates, all ESBL-Ec from food sources were phylogenetically unrelated to ESBL-Ec from CA-UTI patients indicating that MDR UPEC are less likely to have originated from food sources.

T10-11 Impact of the COVID-19 Pandemic on Food Safety Infraction and Pass Rates in Restaurants and Take-Out Facilities in Toronto, Canada

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Introduction: The coronavirus disease (COVID-19) pandemic resulted in major disruptions to the food service industry in 2020, including temporary closures of indoor dining, a shift to providing take-out meals, and implementation of enhanced infection control measures to prevent COVID-19 virus transmission.

Purpose: The objective of this study was to conduct an interrupted time series analysis to investigate the impact of the COVID-19 pandemic on food safety inspection trends in Toronto, Canada.

Methods: Inspection data were obtained from the City of Toronto from Jan. 2017 to Aug. 2022 and ordered as a weekly time series. Segmented regression was conducted using a Bayesian framework to evaluate weekly infraction rates (per number of inspections conducted) and inspection pass rates (vs. conditional pass or fail outcomes). These two outcomes were modelled as negative binomial and binomial regression models, respectively. Both models included time, the week of pandemic declaration (Mar. 17, 2020), and their interaction as fixed effects, and month as a varying effect. The final dataset included data on 77,019 inspections over 280 weeks during the study timeframe.

Results: On average, a 0.33-point lower weekly infraction rate (95% credible interval [CI]: 0.26, 0.41) and a 1.8% higher probability of passing food safety inspections (95% CI: 1.0%, 2.6%) were found in the pandemic period compared to before the pandemic. Models predicted much lower infraction rates and higher pass rates immediately following the pandemic that are regressing back toward pre-pandemic levels in 2022. Seasonal effects were also identified, with infraction rates highest in April and inspection pass rates lowest in August.

Significance: The COVID-19 pandemic resulted in an initial positive effect on food safety outcomes in restaurants and take-out food establishments in Toronto in 2020, but this effect appears to be temporary. Additional research is needed on long-term inspection trends post-pandemic, and to further investigate noted seasonal inspection trends.

T10-12 Investigating Socio-Environmental Inequities in the Consumption of Unsafe Food and Water in Canada

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Introduction: Foodborne and waterborne diseases are preventable diseases affecting over 4 million Canadians annually. This burden is inequitably borne by certain socio-environmental populations. Due to a lack of routinely collected data, the extent of socio-environmental inequities is poorly understood.

Purpose: Our objective is to summarize knowledge on socio-environmental inequities in foodborne and waterborne diseases in Canada, including socio-environmental stratifiers used and sub-populations and geographic regions where inequities have been described.

Methods: We conducted a scoping review to identify relevant Canadian academic and grey literature. A comprehensive search strategy of keywords and MeSH terms of relevant foodborne and waterborne diseases, biological and chemical contaminants, and Canadian geography was developed and applied to academic and grey literature. Citations were deduplicated and screened by two independent reviewers in two stages, first screening titles and abstracts and then screening full texts, against study inclusion criteria. Data from full text articles was extracted and summarized according to socio-environmental stratifiers.

Results: Our initial search yielded 10795 articles. Thirty-five articles were identified for data extraction. Several socio-environmental stratifiers were evaluated, including age (34), place of residence (28), gender/sex (28), seasonality (13), socioeconomic status (7), Indigenous status (3), and race/ethnicity (2). Inequities by language, occupation, religion, and social capital were not studied. The most commonly studied diseases or contaminants were salmonellosis (6), cryptosporidiosis (4), Escherichia coli (4), and mercury (4). Study locations were distributed across Canada, including: BC (3), Alberta (4) Manitoba (2), Ontario (6), Quebec (6), Newfoundland and Labrador (1), PEI (1), NWT (3), Nunavut (1), and multi-province (8).

Significance: My research is the first to synthesize evidence of foodborne and waterborne disease inequities in Canada using a broad range of socio-environmental factors. Anticipated impact includes motivating new areas of research, adding high-quality evidence to inform public health action, and ultimately reducing inequities by tailoring prevention strategies.

T11-01 Microbial Dynamics in Mixed-Culture Biofilms of *Salmonella* Typhimurium and *Escherichia coli* O157:H7 and Bacteria Surviving Sanitation of Conveyor Belts of Meat Processing Plants

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Introduction: Biofilms are recognized for their role in recurring contamination of meat with foodborne pathogens including *Salmonella* Typhimurium and *Escherichia coli* O157:H7. Biofilm formation by pathogens is affected by their inherent biofilm-forming capabilities and environmental factors including the companion microbiota.

Purpose: To determine the impact of meat processing surface bacteria (MPB) on biofilm formation by *E. coli* (non-biofilm former) and *S*. Typhimurium (strong biofilm former).

Methods: MPB were recovered from the contacting surface (CS), non-contacting surface (NCS), and roller surface (RS) of a beef plant conveyor belt after sanitation. *E. coli* and *S*. Typhimurium were co-inoculated with MPB (CO) or after a delay of 48 h (IS) into biofilm reactors containing stainless steel coupons and incubated at 15°C for up to 144 h. Coupons were withdrawn at 2, 48 and 144 h (CO) or 2, 48, 50 and 144 h (IS) and analyzed by conventional plating method and 16S rRNA gene amplicon sequencing.

Results: The total bacterial counts in biofilms reached approximately 6.5 log CFU/cm^2, regardless of MPB type or development mode. The mean counts for O157 and ST under equivalent conditions mostly did not differ ($P>0.05$), except for the IS set at 50 h where no O157 were recovered. *E. coli* and *S*. Typhimurium were 1.6±2.1% and 4.7±5.0% (CO) and 1.1±2.2% and 2.0±2.8% (IS) of the final population. *Pseudomonas* dominated the MPB inocula and biofilms, regardless of MPB type or development mode.

Significance: Mixed-species biofilms of post-sanitation equipment microbiota can assimilate both biofilm-forming and non-biofilm-forming strains of pathogens. Thus, the findings suggest sanitation focusing on pathogens as well commensal bacteria on equipment would be necessary to avoid pathogen persistence in food processing environment.

T11-02 The Management of *Salmonella* Enteritidis in New Zealand's Commercial Poultry Flocks after a 2021 Incursion

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Introduction: New Zealand (NZ) commercial poultry flocks were considered free of *Salmonella* Enteritidis (SE) until March 2021, when SE sequence type (ST) 11, phage type (PT) 8, was detected at slaughter during routine testing. The isolate was genomically linked to ongoing human cases as well as a restaurant outbreak dating to 2019.

Purpose: An emergency response was launched by New Zealand Food Safety, with support from poultry industry bodies, to determine the extent of the incursion across commercial poultry farms and manage the risks of SE infections.

Methods: The ongoing response included investigation of live poultry breeders, growers, and transport routes, whole genome sequencing of human and veterinary SE isolates, and intensive environmental testing of 25 farms representing 80% of the national egg supply. An emergency control scheme implemented to, among other measures, enhance on-farm biosecurity, improve cleaning and disinfection protocols, and mandate vaccination and environmental testing.

Results: Three farms were found to be positive for the SE outbreak strain and all had direct or indirect links to a single hatchery, which also tested positive for the outbreak strain. As part of the response, raw product from subsequent positive flocks was prevented from entering the human food chain. Intensive environmental testing after depopulation, cleaning and disinfection of positive sheds and continued environmental testing continues to give some confidence that SE is no longer at detectable levels on these farms.

The NZ poultry industry is being supported through a transition phase to include *Salmonella* as part of commercial poultry farms' risk management programmes.

Significance: Monitoring of the entire poultry chain for SE continues. Some sporadic human cases of the outbreak strain have been identified in 2023. The incursion may have been detected early enough to prevent long-term establishment and further spread of SE. The increased biosecurity and continued testing will contribute to preventing another incursion.

T11-03 In-Feed Supplementation of Linalool Reduces *Salmonella* Enteritidis Colonization in Broiler Chickens

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* Enteritidis (SE) is a major foodborne pathogen with chickens serving as a reservoir host. Effective, non-antibiotic-based, on-farm strategies for reducing SE colonization in chickens are critical for enhancing the microbiological safety of poultry products.

Purpose: This study investigated the efficacy of in-feed supplementation with Linalool (3,7-Dimethylocta-1,6-dien-3-ol), a “generally recognized as safe” (GRAS) compound in reducing SE colonization in broilers.

Methods: Day-old chicks (n=212/experiment) were assigned to six treatment groups (n=24/treatment group): negative control (no SE, no linalool), three compound control groups (no SE, fed with 1%, 1.5%, 1.8% [v/w] linalool), positive control (SE, no linalool), and three compound treated, SE challenged groups (SE, fed with 1%, 1.5%, 1.8% linalool). On day 7, birds receiving SE challenge were inoculated with □8.0 log CFU of a four-strain cocktail of SE, and checked for cecal colonization (n=5 chicks/experiment) after 48 h. Eight birds from each treatment group were euthanized and necropsied on days 14, 24 and 34 for enumerating SE populations in the cecum, spleen and liver. Additionally, the in vitro effect of linalool on SE colonization genes was investigated by RT-qPCR. The experiment was replicated twice, and the data were analyzed by two way-ANOVA.

Results: In-feed supplementation of linalool (1.5% and 1.8%) for 35 days decreased SE counts in the cecum by at least 1.5 to 2.0 log CFU/g ($P<0.05$). Furthermore, SE persistence in the liver and spleen in linalool-supplemented birds was lower than that in controls ($P<0.05$). No significant difference was observed in the body weight and histopathological scoring among birds in the treatment groups. RT-qPCR data revealed that linalool downregulated the expression of SE colonization genes ($P<0.05$).

Significance: Results suggest that in-feed supplementation of linalool is effective in reducing SE colonization in chickens.

T11-04 Assessing *Salmonella* Serovar Dynamics through Broiler Processing

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Introduction: Nearly one in five salmonellosis cases are attributed to broilers, with renewed efforts to improve *Salmonella* surveillance during broiler production and processing. A limitation to *Salmonella* surveillance is that 1-3 colonies are picked for confirmation and characterization, favoring isolation of the most abundant serovar found in a sample, while low abundance serovars often remain undetected.

Purpose: The objective was to determine how processing interventions influence serovar population dynamics by deep serotyping *Salmonella*-positive broiler carcasses collected at hot rehang and post-chill.

Methods: Paired hot rehang and post-chill broiler carcasses were collected by USDA-FSIS from processing establishments across the U.S. from August-November 2022. CRISPR-SeroSeq, a high-resolution sequencing approach that quantifies the relative frequency of multiple serovars present in a sample, was used to deep serotype all *Salmonella*-positive hot rehang (n=86) and post-chill (n=19) samples.

Results: This data set included 19 paired hot rehang and post-chill carcass rinses and 67 unpaired hot rehang carcass rinses (n=105). Multiple serovars were detected in 41.0% (43/105) of samples, the greatest proportion being from rehang samples (95.3%; 41/43). Rehang carcasses contained more serovars per sample (1.6 serovars/carcass, range 1-5 serovars/sample) than post-chill carcasses (1.1 serovars/sample, range 1-2 serovars/sample)(Mann Whitney U, p=0.00932). Sixteen different serovars were identified. The most common identified in hot rehang carcasses were serovars Kentucky (73.3%; 63/86), Infantis (34.9%; 29/86), and Typhimurium (18.6%; 16/86). The predominant serovar detected in post-chill samples was also Kentucky (89.5%; 17/19). Serovars Enteritidis (15.8%; 3/19) and Typhimurium (5.3%; 1/19) were the only other serovars detected in post-chill carcasses. There was 99.0% concordance between serovars called by CRISPR-SeroSeq and whole genome sequence serotyping of single isolates.

Significance: These data demonstrate that processing interventions are effective in reducing *Salmonella*, as serovar complexity was reduced in nearly all post-chill samples. Deep serotyping is a powerful technique for profiling *Salmonella* populations in broiler carcasses.

Technical

T11-05 Implementation of Machine Learning and Multi Spectral Imaging in Assessing Poultry Spoilage

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Introduction: Meat management safety and/or quality system is probably the main challenge of the agri-food industry that is expected to be addressed in the current environment of tremendous technological progress.

Purpose: Spectral/imaging data combined with machine learning approaches might stand as promising alternatives for screening a large amount of data accompanied by the official methods (e.g., microbiological) for verification purposes or for ambivalent samples

Methods: In total 762 poultry samples were analyzed. Three batches were used for the external validation (n=165), while the rest of the data (n=597) were used for training and testing the model (80%-20%). The partitioning schema was selected so that the different batches, storage conditions (i.e., temperature, packaging), and different years of the purchase were included in both the training set and the test set of the models. Partial least squares regression (PLSR) was applied for model development and evaluation. The data analysis was implemented in R and Rstudio using the package ‘pls.

Results: PLSR models were trained, tested, and externally validated. The R2, RMSE for the test set and external validation were 0.62, 0.88 (test set) and 0.61, 0.88 (external validation), respectively. The performance was similar in both cases which indicates the robustness of the model. MSI data showed potential for the estimation of microbiological data considering the complexity of microbiological spoilage and the variability has been inserted in the models. In the future has been planned the investigation of a pixel-wise analysis and exploration of each parameter has been inserted in the model separately (e.g., storage temperature).

Significance: Handling and processing big data derived from various storage conditions can provide useful information for real-time decision-making. This work has been funded by the project DiTECT (861915).

T11-06 Cultivation-Dependent and Cultivation-Independent Methods Reveal the Bacterial Ecology of Vacuum-Packed Beef Meat with Different pH during Chilled Storage

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Introduction: The spoilage of vacuum-packed beef, before reaching the expected shelf-life, leads to the loss of meat quality attributes that could affect the Brazilian national and international markets.

Purpose: This research studied the growth of spoilage populations in vacuum-packed meat samples from three types of cuts with ranges of pH, 5.4 to 5.8, 5.8 to 6.1 and > 6.1 stored at 0˚C, 4℃ and 7˚C.

Methods: Culture-dependent methods were used for microbial enumeration for a maximum period of storage of 49 days. In addition, 16 Sr DNA gene

sequencing and Headspace solid-phase micro-extraction along with Gas chromatography-mass spectrometry were used to support microbiological and volatiles analyses, at the beginning (0 days) and the end of the storage period, at 49 days (0℃), 41 days (4℃), and 42 days (7℃).

Results: Lactic acid bacteria showed the highest counts (>6.0 log CFU/g), regardless of the studied conditions. *Enterobacteriaceae* grew in beef with pH<5.8 stored from 0°C to 7°C. Beef with pH>5.8 and higher storage temperature led to higher counts of these bacteria (>5.0 log CFU/g). *Brochothrix thermosphacta* remained mostly below the quantification limit in meat with pH 5.4 to 5.8, contrary to the behaviour observed in meat with pH>5.8 (~3.0 log CFU/g). The genera *Lactobacillus*, *Lactococcus* and *Leuconostoc* predominated in all samples. *Carnobacterium, Serratia, Hafnia-Obesumbacterium,* and *Clostridium stricto sensu 5*, among others, were notably involved in meat deterioration. These populations presented significant correlations (FDR<0.05) with 11 volatile organic compounds (VOC).

Significance: This work shows that lactic acid bacteria was the dominant microbiota no matter the pH of the beef cuts, pH values and storage temperature and that the pH of the beef cut, and storage temperature were critical for the bacterial ecology of beef cuts.

T11-07 The Microbiota in Lymph Nodes of Cattle Presented for Slaughter in a Canadian Meat Processing Plant

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Introduction: Lymph nodes (LNs) harboring pathogens, when being incorporated into ground beef, may cause food safety risk, however, the information on the microbiota in LNs of cattle is very limited.

Purpose: To investigate the presence of two main foodborne pathogens, *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) and profile the microbiota in LNs of cattle in a federally inspected Canadian meat processing plant.

Methods: We used methods including conventional culture plating, PCR, 16S rRNA gene amplicon sequencing (16S sequencing) and metagenome sequencing to detect/characterize microbiota in LNs. LNs at two anatomical locations, subiliac and popliteal, from 80 cattle including both cows (n=34) and steers (n=46), were included in this study. Hides from the same cattle were also included for the detection of *Salmonella* and STEC.

Results: A similar proportion of both LNs (88.8%, subiliac; 86.2%, popliteal) were found to harbor bacteria, whereas a larger percentage of popliteal LNs than subiliac LNs had *Enterobacteriaceae* (EB) (popliteal, 67.5%; subiliac, 42.5%) and coliforms (popliteal, 50%; subiliac, 31.2%) detected (Fisher's exact test, P<0.05). Also, popliteal LNs (2.4 log CFU/g) had a larger bacterial load than subiliac LNs (1 log CFU/g) when bacteria were detected in both LN types from the same cattle (T-test, P <0.05). Neither *Salmonella* or STEC were found in LNs although STEC was detected at a significant percentage on beef hides (50.6%). Both 16S sequencing and metagenome sequencing found the predominance of three genera *Escherichia* (13-34.6% among bacterial community), *Clostridium* (12.6-20.6%) and *Streptococcus* (9.7-10%) in LNs. With metagenome sequencing, *E. coli* (13%), *Clostridium perfringens* (11.1%) and *Streptococcus uberis* (6%) were found to be predominant in LNs.

Significance: This study did not find *Salmonella* or STEC in either popliteal or subiliac LNs in cattle presented for slaughter. However, *C. perfringens* was found abundant in certain LNs, which may cause food safety risk.

T11-08 Non-Destructive Cloth Sampling Method to Replace N60 Sampling of U.S. Beef Trim

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Introduction: In the United States the standard N60 method for sampling beef trim products requires samplers to use hooks and knives to cut 60 pieces of raw meat that may be analyzed for pathogens.

Purpose: FSIS conducted studies to determine if a non-destructive cloth sampling method is fit to replace N60 for regulatory sampling of domestic beef trim products.

Methods: In addition to in-lab studies for STEC, FSIS field personnel collected cloth samples paired with routine N60 verification samples of beef manufacturing trimmings. It was important to add a buffer to cloth samples before shipping to ensure adequate organism recovery. Paired samples were analyzed for *Salmonella* presence and aerobic counts. Differences in *Salmonella* percent positive were evaluated by McNemar's Chi-square.

Results: There was no significant difference in *Salmonella* percent positive detected by cloth (45/2578; 1.7%) or N60 (52/2578; 2.0%) (p=0.37; CI_{95}: -0.8% to 0.2%). Cloth sampling recovered 0.38 log CFU/sample more aerobic bacteria than N60 sampling on average (cloth mean 4.72 log CFU/sample; N60 = 4.34 log CFU/sample). Cloth sampling is faster and less expensive than N60.

Significance: Beginning February 1, 2023, FSIS will switch to cloth sampling method for domestic beef manufacturing trimmings and bench trim for adulterant Shiga toxin-producing *Escherichia coli (E. coli)* (STEC) and *Salmonella*.

T11-09 FTIR-ATR Spectroscopy for the Assessment of Microbiological Quality of Meat

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Introduction: The rapid and accurate food quality control is considered a crucial matter for industries. Therefore, it's significant to develop new methods for the prediction of food spoilage.

Purpose: The main aim of this study is to predict microbiological quality of meat (beef, chicken) being in different states (fresh, frozen) using FTIR-ATR instruments coupled with multivariate data analysis.

Methods: Two batches of each meat species, chicken (n=135) and beef (n=163) were provided by the food industries and stored under aerobic and vacuum packaging, respectively. The storage temperatures were 0, 4, 8°C for both food commodities and 12°C for poultry samples. The fresh samples were microbiologically analyzed for the enumeration of total viable counts (TVC) and spectral data were acquired at the same time intervals using $FTIR_1$. The samples were stored at -20°C and thawed after about one-two months. Spectral data were collected once again using two instruments ($FTIR_1$, $FTIR_2$). Stratified sampling was applied so as 70% of each dataset to be used for training and 30% for testing the models. Partial least squares regression (PLS-R) was applied on the collected data for the prediction of microbiological counts after the spectral data were preprocessed.

Results: The prediction performance in terms of R^2 and RMSE of the test set were 0.69, 0.82 ($FTIR_1$) for the fresh chicken samples, respectively. The performance for the frozen samples case were R^2=0.51, RMSE=1.05, ($FTIR_1$) and R^2=0.68, RMSE=0.85 ($FTIR_2$). The respective performance of beef samples for $FTIR_1$ (fresh: R^2=0.36, RMSE=0.94, frozen: R^2=0.44 RMSE=0.86) and $FTIR_2$ (frozen: R^2=0.29 RMSE=0.89) was inferior.

Significance: The collected information from this multifactorial study will be further investigated in terms of important features and prediction of the microbiological quality independent of the samples' state (fresh, frozen) and FTIR-ATR instrument. This work has been funded by the project DiTECT (861915).

T11-10 Utilization of Lauric Arginate as a Surface Antimicrobial in Fresh Pork and Microwave Cooked Bacon

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Introduction: Lauric arginate (LAE) is an effective surface antimicrobial used by meat and poultry industry to mitigate pathogens including *Listeria.* There is very limited research on the efficacy of LAE as a control intervention for recontamination and subsequent growth of *L. monocytogenes* on cooked bacon. Additionally, utilization of LAE on fresh pork for *Salmonella* mitigation has not been studied widely.
Purpose: Objective of this study was to determine the inhibitory activity of LAE on *Listeria monocytogenes* inoculated microwave cooked bacon bits and *Salmonella* inoculated fresh pork tenderloins at 4°C.
Methods: Microwave cooked RTE bacon bits (n=6, 50 g) and raw pork tenderloins (n=20, 100 g) were inoculated with a 6-strain *L. monocytogenes* (10^7 CFU/g) and 4-strain *Salmonella* (10^6 CFU/g) cocktail, respectively. Both inoculated and un-inoculated samples were surface applied with 44, 100, and 200 ppm of LAE. Bacon and tenderloin samples were packaged under 100% Nitrogen and vacuum, respectively, and stored at 40°F (4.4°C). Inoculated samples were analyzed over a 30-day period by direct plating on MOX and XLD to enumerate *L. monocytogenes and Salmonella*, respectively, and un-inoculated samples were analyzed for total aerobic counts.
Results: At 4°C, *L. monocytogenes* growth was not observed on cooked bacon with or without LAE by 30 days. The detection limit was 40 CFU/g. *L. monocytogenes* populations in bacon samples with 44 and 100 ppm LAE were reduced by 0.25 log CFU/g. However, there were no significant differences in populations between samples with 44 and 100 ppm LAE (P>0.05). After 3 days at 4°C, one-log reduction in *Salmonella* and 0.25 log reduction in total aerobic bacteria was observed on pork tenderloins with 200 ppm LAE.
Significance: LAE is a potential *Salmonella* intervention on fresh pork cuts. However, it is not very effective in reducing *L. monocytogenes* on cooked bacon. Findings suggest cooked bacon doesn't support *L. monocytogenes* growth at 4°C.

T11-11 Can HPP be Used to Manufacture Safe Hams with Reduced Preservatives?

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❖ ***Developing Scientist Entrant***

Introduction: Reduction or replacement of preservatives is a major challenge for the safety and shelf-life of meat products. Although HPP has shown promise in enhancing the safety of preservative-reduced meat products, the recovery of HPP-induced sub-lethally injured cells during storage is still a major challenge.
Purpose: Evaluate the effectiveness of HPP in enhancing the safety of ham formulations with reduced level buffered vinegar and sodium diacetate.
Methods: Ham formulations inoculated with *Listeria monocytogenes* or *Salmonella* Enteritidis at approximately 6 log CFU/g containing different levels of buffered vinegar (0, 1.1% and 2.2% w/w) or sodium diacetate (0, 0.65% and 1.3% w/w), were subjected to pressures of 0, 400 and 600 MPa for 3 min and then stored at 4 and 7°C for up to 28 days. Samples were analyzed for *L. monocytogenes*, and *S.* Enteritidis counts at predetermined time intervals by plating on selective media. One-way ANOVA test was used for statistical analysis and Tukey's test was performed to significant differences in log-reductions between formulations, HPP treatments, storage temperatures and time.
Results: Reduction in preservative levels significantly increased *L. monocytogenes*, and *S.* Enteritids counts after 14 and 28 days of storage at 4 and 7 °C, respectively in all non-pressurized samples. The efficacy of HPP at 400 MPa to reduce *L. monocytogenes* and *S.* Enteritids was unaffected by preservative levels during storage at 4°C. HPP at 400 MPa significantly improved the effect of preservatives on Listeria at day 28 of storage at 7°C. HPP at 600 MPa for 3 min reduced *L. monocytogenes* and *S.* Enteritids to below detectable levels in all ham formulations stored at 4°C. However, hams without preservatives showed detectable *L. monocytogenes* counts at day 28 stored at 7°C
Significance: HPP at 600 MPa for 3 min can be successfully used to enhance the safety of preservative-reduced hams.

Technical

T11-12 Impact of Operational Parameters on Pathogen Lethality for Dry Fermented Sausages

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❖ ***Developing Scientist Entrant***

Introduction: Limited data is available regarding pathogen reduction during the manufacture of fermented and dried ready-to-eat (RTE) sausages, particularly for the influence of diameter on drying time and inactivation.
Purpose: This study aimed to determine the optimal number of drying days to accomplish the required pathogen reduction for a range of product diameters.
Methods: Salami, representing a fermented and dried RTE sausage, was used for the study. The sausage batter was formulated using a modified recipe from Michigan State University Meat lab with CHR. Hansen starter culture, SafePro® B-LC-007. Batter was inoculated with five-strain cocktails of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* at a concentration of 3×10^7 CFU/g sausage. Then, the inoculated batter was stuffed into different size casings. Inoculated sausages were fermented to pH 4.8 ± 0.1 and dried for 30 to 60 days. Pathogens from the sausages were enumerated and pH & Aw were measured during drying. Small sausages (18 and 30 mm) and large sausages (60, 90, and 110 mm) were sampled every third day over 30 days and every sixth day over 60 days, respectively. Two independent trials were conducted with two replicates per trial.
Results: An inverse correlation between sausage size and the pathogen log reduction was observed, larger diameter sausages resulted in lower pathogen reduction. For the smaller sausages (based on Trials 1 & 2), 21 ± 3 days and 24 ± 3 days of drying for the 18mm and 30mm sausages, respectively, were required to achieve a five-log reduction of all the pathogens. For the large sausages (based on Trial 1), 48 days of drying were required.
Significance: This study contributes to ensuring food safety by advising fermented sausage producers on the optimal number of drying days to accomplish the required pathogen reduction for different sausage diameters.

T12-01 Moving Data Forward: Disseminating Real-Time Foodborne and Waterborne Data with the Bacteria, Enteric, Amoeba, and Mycotics (BEAM) Dashboard

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Introduction: The System for Enteric Disease Response, Investigation, and Coordination (SEDRIC) is a customized cloud-based software integrating multiple data sources, including PulseNet, related to enteric disease surveillance and outbreak response used by the Centers for Disease Control and Prevention and its partners.
Purpose: To address partner needs and inform prevention efforts the Division of Foodborne, Waterborne, and Environmental Diseases (DFWED) developed the BEAM Dashboard, an interactive dashboard using data from SEDRIC.
Methods: Informal, open-ended discussions with over thirty individuals representing a range of partners including academics, regulatory partners, industry, trade associations, retail companies, and consumer groups informed a multiphase plan for dashboard development. The dashboard uses Microsoft Power BI, an enterprise business intelligence tool, to address the challenge of developing an easy-to-use and easy-to-create dashboard. We used a custom Open Database Connectivity (ODBC) driver to pull data from the SEDRIC database to Power BI. Interactive bar, line, doughnut charts, and maps were generated using the Power BI inbuilt visualization tools. Slicers were added to let the end-users filter data by years and geographical regions.

Results: Partner needs fell into six categories: data linkages with automated analytics, data downloads, user training, customized programmable alerts, direct application programming interfaces (API) with industry to inform prevention efforts, and future linkages with any available regulatory data to support farm-to-fork analyses. The initial version of the dashboard provides real-time aggregated *Salmonella, Shigella*, and Shiga toxin-producing *Escherichia coli* serotype-specific information. It was published in May 2022 and received 1750+ views within a month. The dashboard lets the users interact using point and click actions to filter variables and visualize complex data from different perspectives.

Significance: The BEAM Dashboard improves data accessibility and utility. Additional benefits of this endeavor include expanding and normalizing participation in data sharing and use of DFWED prevention analytic services.

T12-02 Efficacy of Preharvest Water Treatments for Reduction of Foodborne Pathogens in Surface Water

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Developing Scientist Entrant

Introduction: Contaminated pre-harvest irrigation water can be a major transmission route for foodborne pathogens in fresh produce production. For these reasons, treatment of preharvest agricultural water has drawn attention as a risk management strategy, but data are lacking regarding efficacy of sanitizer treatments using water quality representative of that typically used for production.

Purpose: To evaluate the efficacy of commercially available sanitizer treatments at different concentrations under various parameters to reduce foodborne bacteria in preharvest agricultural water.

Methods: Surface water collected from two sources in Georgia was inoculated with a rifampicin-resistant cocktail of seven serovars of STEC or *Salmonella enterica* (~9 to 10 log CFU/ml). The water was equilibrated at 12°C and 32°C and then treated with free chlorine (at 4 ppm and 10 ppm) derived from calcium hypochlorite tablets or peroxyacetic acid (PAA) (at 6 ppm and 10 ppm) at a contact time of 5 and 10 minutes. The sanitizer was then neutralized with sodium metabisulfite. Inoculated water without any sanitizer added was used as a control treatment. Thirty-two treatments with two biological replicates and three samples per replicate were evaluated (n=192).

Results: All the treatment combinations resulted in $\geq$ 4.73 log CFU/ml reduction in both water sources (limit of detection 0.9 log CFU/ml). Lower concentrations of free chlorine and PAA at 12°C resulted in the lowest log reduction ($P<0.05$) for both pathogens. At higher sanitizer concentrations, the temperature had no significant influence ($P>0.05$) on bacterial log reduction. Free chlorine at 10 ppm for 5 minutes at 32°C caused the highest log reduction for STEC (6.34 log CFU/ml), while PAA at 10 ppm for 10 minutes at 32°C caused the highest reduction for *S. enterica* (6.27 log CFU/ml).

Significance: This study highlights the effectiveness of commercial sanitizers under different temperature and contact time conditions for bacterial reduction in pre-harvest irrigation water.

T12-03 Long-Term Surveillance Shows a High Prevalence and Diversity of *Salmonella* spp. in Surface Waters Used for Food Production in Brazil, Chile, and Mexico

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Introduction: Surface water is a primary source of irrigation for crops and a potential vehicle for *Salmonella* contamination.

Purpose: To describe the prevalence and diversity of *Salmonella* in surface waters from Brazil, Chile, and Mexico.

Methods: Surface waters were obtained from Chile (n=2039), Mexico (n=689), and Brazil (n=539) from 2019-2022. Samples were *in-situ* filtered (10L) through modified Moore Swabs and processed according to modified *Salmonella* FDA/BAM procedures. Genomes (1 to 10 isolates per positive sample) were sequenced at CFSAN/FDA on Illumina platforms. Genomes were analyzed for MLST/sequence type (ST) and cgMLST in Ridom Seqsphere+, and serotypes were predicted with SISTR and SeqSero. Phylogenetic relationships were visualized in a minimum-spanning tree on Ridom.

Results: Salmonella was present in 45.8% of samples (1495/3267). Brazil showed the highest prevalence (70.9%), followed by Mexico (63.4%) and Chile (33.2%). Genomes (n=1541) were selected from Mexico (n=686), Chile (n=592), and Brazil (n=263) for analysis; 156 STs were identified, and ST 32 (7.2%), 13 (5.2%), and 19 (5.2%) were the most common. Multiple serotypes (104) were identified, and 18 were common for all countries. Among them, Newport was the most frequent (15.5%;162/1541), followed by Typhimurium (7.85%; 120/1541) and Infantis (7.4%; 114/1541). Meanwhile, most Enteritidis were from Chile (95.6%; 43/45). Mexico showed the highest serotype variability (n=61), followed by Chile (n=53) and Brazil (n=49). About 60.6% (63/104) of serotypes were found in a single country; however, they only represented 20.4% of genomes (315/1541). cgMLST included 1251 genes, and genomes were grouped in 231 clusters. While most clusters (97.4%) comprised genomes of the same serotype and country, mixed-country clusters were detected for Enteritidis, Typhimurium, Agona, Soerenga, Corvallis, and Infantis.

Significance: Surface waters are important reservoirs of epidemiologically and clinically significant *Salmonella* serotypes in Latin America. Research is required to better understand *Salmonella* ecology and epidemiology in surface waters.

T12-04 Identifying Risk Zones of Irrigation Water Contamination in Central Chile: A Collaborative Work between Agricultural Producers and Academia

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Introduction: Since 2018, we have been working with agricultural producers to learn about their food safety problems. Farmers from different areas share the same opinion that water pollution with fecal material and pathogens is their most important concern.

Purpose: A total of 240 water samples were collected in 45 sampling sites located in the VI region of Chile to determine the biological quality of irrigation water used by agricultural producers. Samples were collected monthly for five months during the 2019-2020 and 2022-2023 irrigation periods (October to March)

***Methods**:* Sampling sites were selected using a risk mapping methodology in which producers pinpointed sources of water contamination. Sample sites corresponded to irrigation canals, streams, and basins. *Salmonella* isolation was carried out using the modified BAM methodology and confirmed PCR using the invA gene. Fecal coliforms and *E.* coli (FIB) counts were determined using mFC agar and Chromocult® agar media, respectively, and were compared with the Chilean and US EPA surface water quality standards.

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Results: Salmonella was detected in 45% (75/165) of the water samples collected, while 62% (103/165) and 99% (163/165) of the water samples did not comply with the Chilean water quality standards (<1,000 Fecal coliforms/100ml) and the US EPA standards (<126 *E. coli*/100 ml), respectively. Water contamination was more frequently detected in sites located closer to the farms of the producers.

Significance: Salmonella and high levels of fecal contamination was found in irrigation water used by agricultural producers, demonstrating that their concerns were legitimate. Our results highlight the need to work with producers to learn about their problems and develop mitigation and control strategies accordingly to their needs.

T12-05 The Formation of *Salmonella* spp. Biofilms in Drip Tape Commonly Used for Irrigation of Produce

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Developing Scientist Entrant

Introduction: Biofilms are known to form inside drip tape irrigation lines to the point of complete blockage from the emitter holes. However, there is inadequate understanding of how foodborne pathogens may survive and what role biofilm formation may play in these conditions.

Purpose: In this study, we evaluated the formation of *Salmonella* spp. biofilms within an irrigation drip tubing commonly used during produce harvesting.

Methods: Drip-line tubes (without emitters) commonly used for irrigation (1.27 cm internal diameter) were aseptically cut into 60 cm lengths (n=24). Each tube was filled with 100 ml surface water inoculated with 6 log CFU/ml population of a four-serotype cocktail Rifampicin resistant *Salmonella* (Montevideo, Poona, Newport and Enteritidis). Water-filled tubes were incubated at temperatures commonly found in the field 21.1°C for 9 days. *Salmonella* population in the water and attached to the tubing was determined on days 0, 3, 6, and 9. Attached bacteria were dislocated from the tubing by sonication: 2 min at a 70W ultrasonic power. Parts of a tube sample were analyzed with confocal imaging using a combination of SYTO9 and SYTOX red staining solution.

Results: Salmonella population decreased progressively by 1 log CFU/ml from the day of inoculation until day 9 in the water samples. Cross-contamination from the water to the drip tubing was observed. *Salmonella* population on the tubing increased by 3 log CFU/ml from day 0 to day 3 ($P<0.05$) and remained constant (~ 7 log CFU/ml) until day 9. Confocal imaging confirmed the presence of extracellular DNA.

Significance: This work has established that *Salmonella* can both attach and grow under these static conditions. Future research will evaluate behavior with lower initial populations of *Salmonella* and dynamic temperature and water quality parameters to assess what role biofilms may play as a route of preharvest water contamination.

T12-06 Evaluating Harvested Rainwater Quality for Produce Irrigation

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Introduction: Rainwater harvesting could provide an additional water source for farmers, especially in urban agriculture, yet there is currently low adoption in part because of water quality concerns. Additionally, studies on the microbial quality of harvested rainwater are limited and contradictory.

Purpose: The Baltimore City Urban Agriculture Irrigation Water Quality Project, part of the RRIPER (Rooftop Runoff Irrigating Produce Eaten Raw) program, sought to compare harvested rainwater quality with municipal water, the most used urban agriculture irrigation water source, and determine the impact of site characteristics and treatments.

Methods: Between 2021-2022 we collected water samples from 16 Baltimore City farms and gardens (10 using harvested rainwater, 5 using municipal water, and 1 using both) from a total of 23 water sources (16 harvested rainwater, 7 municipal water). Participants were asked to complete surveys on site characteristics, irrigation methods, and water concerns. Water samples (n=52) were tested for generic *E. coli* using standard membrane filtration.

Results: The average *E. coli* concentration for all municipal water samples (0.0008 CFU/100mL) was well below the Good Agricultural Practices (GAPs) irrigation water guideline of 126 CFU/100ml. The average *E. coli* concentration for all harvested water samples (47 CFU/100ml) was also below the GAPs guideline, yet five harvested rainwater samples (10%) from two sites exceeded the guideline. Both sites that exceeded GAPs guidelines had never tested their water and had previously noticed issues with their systems (ex. aging roofing materials and noxious odors). 80% (4/5) of sites using harvested rainwater that completed our survey had never had their water tested, compared to 67% (4/6) of sites using municipal water.

Significance: These findings suggest that harvested rainwater could be a safe irrigation water source, yet point to the importance of water testing. As climate change continues to impact water sources, additional research on harvested rainwater quality and treatment is necessary.

T12-07 Food Safety Attitudes and Practices in a Traditional Food Market in Hawassa, Ethiopia

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Introduction: Every day, millions of people around the world purchase food from traditional markets in low- and middle-income countries; although the knowledge, attitudes, and practices (KAP) of vendors and consumers related to food safety and drivers of food purchasing are not well studied.

Purpose: The purpose of this study was to evaluate food safety decision-making of consumers and vendors in traditional markets.

Methods: In July thru August 2022, a cross-sectional survey of food safety knowledge, attitudes, and practices (KAP) of 150 consumers and 150 vendors was conducted in a market in Hawassa, Ethiopia as part of USAID's EatSafe program. Descriptive statistics were used to synthesize participant demographics and KAP. Variables related to household assets were used to compute the Poverty Probability Index to characterize socioeconomic status.

Results: Most fresh vegetable vendors (87%) were women, while both men and women shopped at the market. Women were primarily responsible for deciding what food to buy, purchasing and preparing food for the household. The likelihood to live below the international poverty line of $3.20 USD/day was 26% for vendors and 19% for consumers. Consumers and vendors had similar reasons to choose who to purchase food from, including price, food quality, vendor personality, and food safety. Consumers used visual and sensory cues to assess food safety and did not commonly discuss food safety with vendors. Vendors were generally not concerned about foodborne disease (73%) but expressed interest in learning about food safety, including how consumption of unsafe food can lead to foodborne illness.

Significance: In traditional markets, consumers and vendors make important decisions that can impact the safety of the food they purchase and sell. Findings from this study can help determine the most effective food safety interventions to influence motivation and ability of consumers and vendors to select safer food at the market and enact food safety practices.

T12-08 *Salmonella enterica* Transfer from Cucumbers to Vinyl Gloves to Tomatoes during Handling

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Introduction: Vinyl gloves are classically used for general cleaning, aesthetic non-invasive procedures among other protocols. The SARS-CoV-2 pandemic saw increased use of gloves while shopping, including for selection of fresh fruits during shopping. Published data on *S. enterica* transfer from fruit to glove or from glove to fruit are scarce but are needed to inform risk management decisions.

Purpose: This research quantifies the transfer of *S. enterica* from cucumber to vinyl gloves and then to tomato, as well as *Salmonella* survival on used gloves at 25°C.

Methods: A *S. enterica* cocktail (*S*. Agona, *S*. Montevideo, *S*. Michigan, *S*. Newport and *S*. Typhimurium) was inoculated (7.5 log CFU/ml) on the surface of sanitized cucumbers and dried for 1 h. A gloved forefinger (~ 4 cm^2 surface area) was kept in contact with the inoculated area without pressure for 2, 5, 10 or 30 seconds. The same forefinger was then kept in contact with a tomato surface for 2, 5, 10 and 30 seconds. The vinyl gloves contaminated by 30 s of contact with cucumbers were held at 25 ºC for up to 9 days. *S. enterica* was enumerated after each contact event and on contaminated gloves over 9 days at 25 °C on Tryptic Soy Agar (TSA) plates incubated at 37°C for 18 h.

Results: Contact time influenced the transfer of *S. enterica* to gloves and fruits. About 4.9 log CFU/sample (65.6%) of *S. enterica* were transferred from cucumbers to gloves after 30 s contact. Subsequently 2.7 log CFU/g (55.3%) were transferred from gloves to tomatoes after 30s of contact. *S. enterica* counts on used gloves were 1.7 log CFU/glove after 9 days at 25°C.

Significance: These findings help understand the risks of cross-contamination when gloves are used to handle fresh produce.

T12-09 Challenges and Opportunities Associated with Using Hospitality Operators' Food Safety Data to Complement Official Food Safety Controls

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Introduction*:* The delivery model for official food controls in the UK food service sector is not fit for purpose. Private sector voluntary Third-Party Assurance (vTPA) schemes such as the Red Tractor® Scheme, have shown that they can be a successful part of the delivery model for official food controls. However, in the food service sector, vTPA schemes are not part of the regulatory framework.

Purpose: Reduce the frequency of LA inspections.

Methods*:* As part of the Food Standards Agency's (FSA) Regulating Our Future program, an initial study using focus groups and semi-structured interviews with three Local Authorities (LAs) and one multi-location Food Business Operator (FBO) aimed to assess whether FBOs' food safety data captured in software (RiskProof®) could be shared with LAs to complement official food safety controls.

Results: A retrospective review indicated that LAs were overwhelmed by the amount of data, struggled to interpret it and preferred to be presented with data in a familiar format i.e., an inspection report. A further study, using semi-structured interviews and a working group with Food Standards Scotland (FSS) and LAs, developed new knowledge of what LAs required in a third-party audit report.

Significance*:* Both studies aimed to reduce the frequency of LA inspections of FBO in the food service sector and reflexive interpretation led to the view that the FSA, FSS, and LAs would be better served by a food service sector vTPA scheme, which has been developed. Using semi-structured interviews, current research aims to identify critical success factors and downsides of the food service vTPA scheme Safe to Trade® from the lens of the regulator, FBO and consumer stakeholder groups. This presentation will discuss the challenges and opportunities associated with using FBOs' food safety data based on the studies and current research programme.

T12-10 Assessing the Relationship between Certified Food Protection Managers' Certification Characteristics and Food Inspection Outcomes in Ohio

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Introduction: All Ohio retail food establishments and food service operations that prepare and serve time/temperature controlled for safety (TCS) foods must employ at least one Certified Food Protection Manager (CFPM); there are multiple providers, and formats (e.g., online, in-person) from which individuals can choose to earn certification.

Purpose: To describe how CFPM certification characteristics impact overall inspection results.

Methods: All Risk Level III and IV food establishments regulated by Franklin County Public Health (FCPH) were recruited in September 2021. Data on CFPM training and certification were obtained for each establishment using a supplemental data collection form (Qualtrics) during a standard inspection. Health inspection data from FCPH was matched to Qualtrics data by Facility ID and date. Logistic regression methods were used to identify if CFPM characteristics (e.g., training format, training provider, etc.) were associated with a failed health inspection.

Results: Supplemental data were collected from 1,852 of the 2,040 establishments that were licensed during the study period. The majority (54.56%) of establishments employed one CFPM at the time of the inspection with 25.11% employing none and 20.73% employing more than one. In establishments with only one CFPM employed, most CFPMs received in-person training (63.77%) with a smaller proportion receiving online training (36.13%). Among the 1,001 establishments with only one CFPM employed, 314 (31.34%) establishments had more than seven uncorrected violations, which is considered a health inspection failure. Among establishments with only one CFPM employed, establishments with an online-trained CFPM were 1.59 (95% CI: 1.21, 2.09) times more likely to have a failed health inspection than those with an in-person trained CFPM.

Significance: Initial results suggest that in-person CFPM training may improve compliance with the State of Ohio Uniform Food Code. The improvement of CFPM training can result in fewer failed health inspections, which leads to improving retail food safety which is important for public health.

T12-11 The Use of the Design Thinking Method in the Food Safety Culture Evolvement Process

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Introduction: Design thinking (DT) is a method based on understanding problems with a focus on immersion and identifying the real needs of the group, which could be used to plan interventions based on the Food Safety (FS) culture diagnoses.

Purpose: To analyze the effectiveness of using the DT for the FS-culture evolvement.

Methods: The implementation of the DT follows 5 stages: 1) empathize, in which we assessed the FS-culture using a mixed-method approach already validated for food services (questionnaires, checklist, and participant observation) to evaluate the FS-culture elements (knowledge, commitment, work environment, leadership, risk perception, communication, work pressure, and normative beliefs, and management systems, styles, and process); 2) define, the characterization of the FS-culture based on the triangulated data and interpretation grid as reactive, active or proactive; 3) ideate, the brainstorming

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process was carried out for the generation of ideas for solutions to the FS-culture transformation; 4) prototype, based on the ideas built in stage three; 5) test, the prototype was implemented. After the DT implementation, FS-culture was assessed again. The case study was conducted in two food services (1) 500 meals/day, 39 food handlers, 5 managers; 2) 350 meals/day, 26 food handlers, 4 managers in Brazil. The Brazilian Ethics Committee approved the project under the number 4.845.608.

Results: The prototype in the two food services constituted a video motivating the improvements in the reactive elements. The prevailing FS-culture was characterized as active-to-proactive (2-3) in all the assessments, showing that the DT did not change the prevailing FS-culture (2-3). However, the DT changed proactively (active-to-proactive; 2-3) some FS-culture elements, as knowledge, commitment, and management system, styles, and processes.

Significance: The prototype creation positively impacted the FS-culture elements, however, it did not evolve FS-culture. The process of the DT should be explored as a way to engage food handlers in the food safety culture.

T12-12 Employees Burnout and Food Safety Behaviors in the Restaurant Industry

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Introduction: Employee burnout is known as mental and physical exhaustion prompted by ongoing stress that can lead to a lack of motivation to work. While employee burnout can lead to job dissatisfaction and demotivation in general, the question whether burnout amongst restaurant employees is associated with food safety behaviors in the context of restaurant remains unanswered.

Purpose: The objective of this study is to measure the level of burnout of restaurant employees and affective job commitment and determine the relationship between these factors and employees' food safety behaviors.

Methods: A total of 143 respondents who are currently working in the restaurants in the U.S as non-managerial employees participated in the survey through M-Turk (58% male and 42% female). Descriptive statistics and multiple linear regression were used for data analysis to test the relationships between employees' burnout, affective job commitment as independent variables and food safety behavioral intention (dependent variable).

Results: The assumptions for the regression analysis were tested including normality, constant variance, linearity, and outliers and the results met the requirements, and no assumptions were violated. The resulting model was statistically significant (F=55.302, P<.001), with adjusted explanator power of $r2$ = 0.538. The variables related to employee's food safety behaviors were employee's burnout (β=-.420 for personal burnout, β=-.315 for work related burnout, P<.001) and affective job commitment (β = 0.584, P<.001).

Significance: The results will be valuable to develop strategies to mitigate employees' burnout and increase affective job commitments to improve food safety behaviors in the restaurant industry.

T13-01 Effects of Dietary Yeast Cell Wall Supplementation on Pathogen Colonization, Performance, and Slaughter Characteristics of Broiler Chickens Inoculated with *Campylobacter jejuni* at Day 16

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❖ ***Developing Scientist Entrant***

Introduction: *Campylobacter jejuni* is an important foodborne pathogen, and chickens are recognized as a common reservoir.

Purpose: To evaluate the effects of dietary yeast cell wall (YCW) in their ability to reduce *C. jejuni* intestinal colonization and to affect performance, carcass yield, and carcass contamination after processing.

Methods: A total of 2,240-day-old Ross 708 males were randomly distributed in 64 pens with eight replicate pens/treatment and 35 birds/pen. Each pen was assigned to one of four dietary treatments: negative control, positive control (bacitracin, 50 g/ton), YCW constant dose (400 g/ton), and YCW step-down dose (800/400/200 g/ton in the starter/grower/finisher periods, respectively) and to one of two inoculations: PBS or CJ at 10^3 CFU/ml administered via oral gavage on day 16. Twenty-four broilers per treatment were collected on days 24, 34, and 42 for *C. jejuni* enumeration and prevalence calculation and 80 birds per treatment were processed to determine carcass yield (64 broilers) and to collect carcass rinses (16 per treatment).

Results: All birds inoculated with *C. jejuni* had similar CFU/ml in the cecal contents at days 24, 34 and 42 (P>0.05). At day 24, although non-significant, a two-log reduction in colonization of *C. jejuni* was observed when broilers were fed with higher doses of YCW in the diet. All birds inoculated with PBS were negative for *C. jejuni* after prevalence testing. The diet or type of inoculation did not influence broiler weight, feed intake, or feed conversion ratio (P>0.05). After processing: 1) carcass yield was similar in all treatments (P>0.05), 2) CJ inoculated birds with an unsupplemented diet had lower CFU/ml counts than treatments supplemented with a positive control diet and YCW constant dose diet (P<0.05), and 3) all birds inoculated with PBS were negative for *C. jejuni*.

Significance: Dietary YCW can potentially reduce *C. jejuni* colonization after inoculation when provided in higher doses in the diet.

T13-02 Effect of *Salmonella* Enteritidis and *Salmonella* Kentucky Co-Challenge on Salmonella Colonization of the Broiler GI Tract

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Introduction: The prevalence of *Salmonella* in all steps of poultry production remains a challenge to the industry, and this pathogen causes numerous illnesses each year. One of the top serotypes associated with foodborne illness is *Salmonella* Enteritidis. Another serotype associated with poultry production is *Salmonella* Kentucky, although this serotype causes fewer diseases in humans.

Purpose: The purpose of this study was to examine if one serovar of *Salmonella* spp. could exclude another in the gastrointestinal tracts of broilers.

Methods: There were four challenge treatments: one consisting of challenge with Enteritidis one day after placement, another with Kentucky challenge after placement, a treatment with Enteritidis challenge after placement followed by Kentucky challenge, and a fourth group with Kentucky challenge followed by Enteritidis. Five birds per pen were randomly selected for necropsy weekly, and cecal content was collected to enumerate for *Salmonella*. A nalidixic acid resistant marker strain of Enteritidis was used, and all samples were plated on typical XLT4 and XLT4 supplemented with nalidixic acid to differentiate between the two serotypes.

Results: A difference in nalidixic acid sensitive counts was observed between challenge treatments, with the Kentucky-only challenge group having higher counts than the Enteritidis/Kentucky group (P≤0.026). A difference in nalidixic acid resistant counts was also observed, with the Enteritidis-only challenge group having higher counts than the Kentucky/Enteritidis group (P≤0.04). Additionally, the Kentucky-only challenge group showed higher prevalence of nalidixic acid sensitive counts compared to the Enteritidis/Kentucky group and the Kentucky/Enteritidis group (P≤0.015). Finally, nalidixic acid resistant counts were higher in the Enteritidis-only challenge group compared to the Kentucky/Enteritidis group (P<0.03).

Significance: These results suggest that one serovar of *Salmonella* spp. can partially exclude the colonization of another subsequently introduced serovar and supports the possibility of developing a *Salmonella* surrogate to exclude *Salmonella* spp. from the gastrointestinal tract of broilers.

T13-03 Biomapping of a Commercial Broiler Hatchery and What It Tells Us about *Salmonella* Prevalence and Diversity

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Introduction: *Salmonella* is an important zoonotic bacterial pathogen within the broiler production chain that impacts human health; therefore, management factors within the production chain that impact *Salmonella* ecology must be determined.

Purpose: Bio-mapping commercial broiler hatcheries with a specific focus on *Salmonella* prevalence and diversity based on sample type and facility area will help identify potential control points for *Salmonella* control in flocks before reaching production farms.

Methods: We followed broiler eggs from two breeder flocks within a hatchery facility on two production days, and sampled five hatchery areas (egg inventory, pre-*inovo* set, post-*inovo* set, hatching, and transport) and from each area collected egg-related, air filter, water drain, and bait box samples (n=184 samples). *Salmonella* isolation was performed using the traditional dual enrichment culture method (n=48), serotyped and *Salmonella* diversity within each sample was assessed using CRISPR SeroSeq. To assess potential microbial correlations with culturable *Salmonella*, genomic DNA was extracted, and microbiome analysis was performed using the Earth Microbiome Project protocol (Illumina MiSeq sequencing followed by QIIME 2.0 sequence analysis).

Results: Overall *Salmonella* prevalence was ~26% (48/184), with the highest prevalence observed in the hatch and transport areas (50% for both), as well as in water drain samples (56%). *Salmonella* serotypes were dominated by Kentucky, Gaminara, and Alachua (n=17, 12, 11, respectively). Data identified transport truck floor drains as a potential reservoir for *Salmonella* serotypes of specific concern to human health (Enteritidis, Mbandaka). Additionally, microbiome analyses revealed *Salmonella*-related taxa were significantly enriched in the feces from the chick pads under the newly hatched chicks in the transport crates.

Significance: By identifying potential control points within hatcheries (transport truck water drains, chick pads), this data can help focus intervention efforts to reduce *Salmonella* loads reaching the live production farms, thus increasing the safety of the broiler food chain.

T13-04 Efficacy of PAA and Chlorine Sanitizers to Reduce *E.coli* in Pre-Harvest Agricultural Water Used in the Southwest

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❖ ***Developing Scientist Entrant***

Introduction: Recent *Escherichia coli* O157:H7 foodborne outbreaks linked to leafy greens and contaminated agricultural irrigation water have elicited auditing schemes for fresh produce that mandate the use of agricultural water treatment to minimize risk.

Purpose: The purpose of this study was to assess the efficacy of two commonly used water treatment chemicals, peracetic acid (PAA) and calcium hypochlorite (chlorine) for use in preharvest production to reduce pathogens.

Methods: PAA and chlorine were evaluated at various residual rates 6 and 8 PPM, and 2 and 4 PPM, respectively, against a seven-strain cocktail of Shiga toxin-producing *E. coli* (STEC), in four surface water sources collected from across the southwestern United States (Yuma, AZ, Maricopa, AZ, Uvalde, TX, and Edinburgh, TX). Water samples were inoculated with 10^9 CFU/ml STEC prescribed cocktail, equilibrated at temperatures 12°C or 32°C, allotted a one-minute contact time (CT), and enumerated in triplicate by spread plate technique. PAA was also trialed at a five-minute CT.

Results: For a one-minute CT, all chlorine treatment trials achieved more than a three-log reduction. Log reduction values (LRVs) for chlorine trials at 2PPM ranged from 3.36-5.49 for trials at 12°C and 3.24-6.04 at 32°C. Chlorine at 4 ppm resulted in LRVs of 3.54-6.15 at 12°C and 3.49-5.45 at 32°C. LRVs for PAA trials evaluated at 12°C, for a one-minute CT, ranged from 0.0-0.31 at 6 ppm and 0.03-0.24 at 8 ppm. For 32°C, PAA LRVs were 0.16-0.56 at 6 ppm and 0.04-1.10 at 8 ppm. When the contact time for PAA treatments was increased to five minutes, LRVs from 1.5 to 5.4 were observed.

Significance: Results indicate that chlorine-based sanitizers may easily meet EPA/FDA label requirements of a three-log microbial reduction in various types of agricultural water with only a one-minute contact time. However, results from the PAA tests indicate that a prolonged contact time is needed to meet proposed Subpart E regulations.

T13-05 Extreme Gradient Boosting (XGB) and Random Forest (RF) Guided Machine Learning Prediction of *Acinetobacter* Density in Fresh Produce Irrigation Source Waters

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Introduction: The transmission of *Acinetobacter* spp. (especially *A. baumannii*) known with high antimicrobial resistance and case fatality ratio onto fresh produce has been demonstrated. An artificial intelligent system for *Acinetobacter* density (AD) determination in irrigation source waters (ISW) would be an invaluable preventive option.

Purpose: This study aimed to predict AD in ISW for fresh produce production using XGB and RF.

Methods: AD and physicochemical variables (PVs) data from three ISW in fresh produce producing areas in South Africa monitored via standard protocols in a year-long study were fitted to XGB and RF algorithms. The models' performance was assessed using regression metrics.

Results: The essential PVs of the ISW ranged as 5.05–9.11, 23.0–279.0 mg/L, 0.02–0.27 PSU, 4.74–28.64 °C, 1.0–1244.0 mg/L, 4.00–1312.0 NTU, and 0.52–10.19 mg/L for pH, TDS, salinity, temperature, TSS, turbidity, and BOD, respectively. AD of the ISW ranged from 1.00–4.56 log CFU. While the contributions of PVs differed in absolute values, AD predicted value coverage by XGB (3.1792 log CFU (min: 1.1040 - max: 4.5828)) was better than RF (3.1795 log CFU (1.3563-4.4514) compared with the raw data. Also, XGB (mean-squared-error (MSE) = 0.0059; root-mean-squared-error (RMSE) = 0.0770; R^2 =0.9914) outmatched RF (MSE = 0.0282; RMSE = 0.1679; R^2 = 0.9584) in predicting AD. Both models ranked temperature as first significant factor in predicting AD based on the mean dropout RMSE loss after 1000 permutations. The partial dependence and residual diagnostics sensitivity of the models revealed their efficient AD prognosticating accuracies in ISW.

Significance: XGB model output could be deployed in fresh produce region as smart early warning system to assist farmers in making decision about ISW microbiological quality in minimizing contamination of produce and promote on-farm food safety management and produce sanitation.

T13-06 Soil Nutrient Levels Associated with *Salmonella* Prevalence and *Escherichia coli* and Total Coliform Concentrations on Produce Farms

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❖ ***Developing Scientist Entrant***

Introduction: Soil can be a route of contamination of fresh produce. Growers routinely manage soil nutrient levels, and little research exists on synergistic or antagonistic effects on foodborne pathogens.

Purpose: This study aimed to (i) determine *Salmonella* prevalence, and generic *Escherichia coli* (gEC) and total coliforms (TC) concentration, and (ii) inves-

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tigate macro- and micronutrient levels associated with each microbial target in soil.

Methods: Three produce farms in Virginia were selected from different regions (eastern, western, and northern VA). Farms were sampled four times to capture seasonal distinctions. Five soil samples were collected from 20 plots ($25m^2$) and pooled in equal quantities to form one sample per plot. A total of 240 samples were collected. Samples (25g) were processed for *Salmonella* using a modified FDA BAM method, while samples (5g) were enumerated for gEC and TC using Petrifilm. PCR was used to confirm presumptive *Salmonella*-positive samples using a single gene (*inv*A). Soil nutrients were tested for each plot and evaluated for their association with each microbial target by Bayesian univariable logistic regression with sampling year as a fixed effect and month and plot as random effects in RStudio.

Results: Salmonella prevalence was 4.2% (10/240) in soil samples. Of the ten *Salmonella* positive samples, nine samples (90%) were from one farm in eastern VA. The average gEC and TC concentrations in soil samples were 1.23 (range 0.95-4.01) and 4.21 (range 1.23-7.12) log CFU/g, respectively. Bayesian models yielded regional differences (specifically, farm location) was a strong indicator of *Salmonella* prevalence (MAP=0.01-0.07;PD=0.97-1.00;ROPE=0.00-0.01). While majority of gEC were below the limit of detection (<1 log CFU/g), increases in soil pH impacted the likelihood of detection (MAP=4.87;PD=1.00;ROPE=0.00).

Significance: While there appeared to be trends between soil nutrients and *Salmonella*, upon analyses, it was challenging to disentangle the strong influences of region and time of year for the data.

T13-07 Detection, Survival, and Inhibition of *Listeria monocytogenes* Based on Carrot Cultivar and Soil Sampling Method

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❖ ***Developing Scientist Entrant***

Introduction: Carrots have been linked with 38 foodborne outbreaks. Identifying the source and route of contamination has been recognized as one of the major hurdles in outbreak investigations. Choosing the most robust soil and crop sampling/testing method will positively contribute to pathogen detection.

Purpose: To evaluate the inhibitory properties of 4-carrot cultivars (4CV) against *Listeria monocytogenes* and contrast the recovery efficiency of two different soil sampling schemes in detection of *L. monocytogenes*, *Salmonella*) and Shiga toxin-producing *Escherichia coli* (STEC) in carrot fields.

Methods: Soil Sampling: The Z pattern-(ZP) and randomized sampling methods-(RSM) were evaluated within one acre of land. The area was divided based on sampling approach and assigned randomly. Four independent sampling events, each consisting of 40 samples per method were collected over 45 days. Each 250g sample was used in the extraction process and subsequent pathogen detection following the bioMerieux GENE-UP LMO2, SLM2, and ECO protocols. Minimum inhibitory concentrations for 4CV were used to determine whether pericarp or internal carrot tissue at 7,200 and 3,600-ppm concentrations could inhibit *L. monocytogenes* inoculated at log 6 and 3 CFU/ml over a period of 24h of incubation (37°C).

Results: No significant difference in the recovery/detection of *L. monocytogenes*, *Salmonella* and STEC was determined between RSM (2.5%) and ZP (1.8%) methods ($P>0.05$). The probability of finding the same organism within the field over 45-days was 1%. Sampling date impacted recovery/detection of the selected pathogens ($P>0.05$). Carrot cultivars had a significant effect on inhibition/growth-rates of *L. monocytogenes* ($P>0.05$) (inhibition-rates (cv): red 91.95%, yellow 79.66% and purple 64.47%/growth-rate: orange cv 1.02±0.37). Carrot exocarp slurries inactivated 84.41% of *L. monocytogenes* while internal tissue slurries only 72.96% ($P>0.05$). Greater inhibition of *L. monocytogenes* was observed in low (84.02%) vs high (73.36%) inoculum doses ($P>0.05$).

Significance: Both pericarp and internal carrot tissue from 3CV inhibit *L. monocytogenes*. No clear differences in pathogen recovery/detection were observed between ZP and RSM.

T13-08 Bacteria Intrinsic to Medicago sativa (alfalfa) Reduce Salmonella Growth in Planta

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Introduction: Multiple foodborne outbreaks of *Salmonella enterica* have been linked to alfalfa sprouts and the bacteria actively grow under standard food processing conditions. We investigated the role of the intrinsic bacteria in inhibiting *Salmonella* growth on alfalfa sprouts as a route to biocontrol of this pathogen.

Purpose: The purpose of this study was to determine whether plant-associated bacteria can reduce *Salmonella enterica* colonization and infection of alfalfa sprouts, ideally preventing them from being a source of foodborne illness.

Methods: We isolated plant associated bacteria from alfalfa seeds and sprouts by plating and streaking onto agar plates. Monoclonal isolates of the bacteria were obtained and tested for their ability to inhibit *Salmonella* Typhimurium growth in competition experiments when grown on alfalfa sprouts over six days. Genome sequencing and annotation was used to construct draft genomes of the bacteria isolated in this study using Illumina sequencing platform.

Results: We observed that a cocktail of five plant-associated bacteria could reduce *Salmonella* growth in alfalfa sprouts from ~10^8 CFU/g to ~10^5 CFU/g, demonstrating a protective role of plant-associated bacteria. Genome sequencing revealed that these bacteria were members of the *Pseudomonas*, *Pantoea*, and *Priestia* genus, and did not possess genes that were pathogenic to plants or animals.

Significance: This demonstrates that plant associated bacteria can be utilized to reduce pathogen levels in fresh produce which may be synergistic with other technologies to improve the safety of sprouts and potentially other fresh produce.

T13-09 Commercial Poultry Litter Particulates as a Vehicle for *Salmonella enterica* Contamination in Cucumber Fruit

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Introduction: Recent outbreaks of *Salmonella* have been associated with consumption of cucumbers. Poultry-associated microorganisms from animal operations have been shown to transfer short distances into adjacent agricultural production environments.

Purpose: To investigate the ability of *Salmonella* to colonize and internalize cucumber fruit when applied to blossoms via contaminated poultry litter.

Methods: Cucumber plants (*Cucumis sativus* var. *sativus*) cultivar Marketmore76 (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 or as liquid inoculum dried overnight in a biosafety cabinet. *Salmonella* contamination was introduced via blossoms at ca. 5.9 log CFU/blossom (freeze-dried method) or 3.2 log CFU/blossom (hood-dried method) via contaminated poultry litter (ca. 10 mg applied to each blossom). In total, 30 Marketmore76 plants at the blossom stage were divided into three treatment groups: a negative control group [dosed with untreated poultry litter (PBS or skim milk); n=2] and two treatment groups [inoculated with freeze-dried *S.* Poona litter (n=14) or air-dried *S.* Poona litter (n=14)]. Cucumbers (harvested 4-55 days post inoculation; average weight 298.8 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 test was used to determine significant differences in prevalence of contamination by inoculum method.

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Results: Of the total mature fruit harvested from freeze-dried- (n=24) or air-dried-*Salmonella*-inoculated blossoms (n=37), 62.5% (15/24) or 27.0% (10/37) were contaminated and 8.3% (2/24) or 8.1% (3/37) had *Salmonella* internalized into the fruit, respectively. Prevalence of internal contamination was equivalent when comparing inoculum methods (x^2=0.001, *P*=0.9750). Surface contamination was significantly lower in air-dried versus freeze-dried method (x^2=7.573, *P*=0.0059).

Significance: These results identified poultry litter as a means for *Salmonella* to colonize and internalize mature fruit when introduced to blossoms during pre-harvest.

T13-10 E. coli Survival in an Organic Romaine Lettuce Field Amended with Treated Biological Soil Amendments of Animal Origin in the Southwest Desert, 2021–2022

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❖ ***Developing Scientist Entrant***

Introduction: Vegetables grown close to the ground and fertilized with untreated biological soil amendments of animal origin (BSAAO) such as raw animal manure are at increased risk for microbial contamination. Proper composting/treatment of BSAAOs using a validated method reduces this risk, but cross-contamination during pre-harvest production can introduce pathogens. Side dressing is a common practice to provide nutrients to organic lettuce plants during production.

Purpose*:* A field trial was conducted to investigate survival of *Escherichia coli* inoculated in soils amended with heat-treated poultry pellets (HTPP) or seabird guano pellets (SGP) with one or two applications of side dressings, and transfer to romaine lettuce.

Methods*:* Organic romaine lettuce seedlings were transplanted into triplicate plots of soil amended with HTPP, SGP, or not amended (UN), followed by overhead irrigation using standard industry practices during the 2021-2022 growing season. Treatment groups received one (HTPP-1, SGP-1) or two (HTPP-2, SGP-2) side dressings at 4 to 6-week intervals. Plots were inoculated with rifampicin-resistant *Escherichia coli* (*E. coli*^{rif}) one month prior to harvest. Levels of *E. coli*^{rif} in composite soil samples and on intact mature lettuce heads were analyzed by direct plating and MPN methods. One-way ANOVA with the Bonferroni comparison test were used to compare *E. coli* levels between treatments.

Results*:* A 2-log reduction in *E. coli*^{rif} levels was observed in soils from HTPP-2, SGP-1, and SGP-2 from 0 to 28 days post-inoculation. In contrast, there was no significant reduction of *E. coli* in soils from HTPP-1 and UN plots in comparison with the other treatments (*P*<0.005). *E. coli*^{rif} levels were also significantly higher on lettuce plant samples grown in plots receiving only one HTPP side dressing (*P*=0.040).

Significance*:* Different side dressing types and intervals using treated BSAAOs may impact *E. coli* persistence in soil and transfer to organic romaine lettuce prior to harvest.

T13-11 Risk Factors Associated with Generic *E. coli* Contamination of Fresh Produce Grown in Manure-Amended Soils in Organic Farms

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❖ ***Developing Scientist Entrant***

Introduction: Animal manure is often used to improve soil health and is particularly important in organic agriculture. Therefore, in order to reduce the risk of produce contamination, the time interval between manure application and produce harvesting still needs further scientific assessment.

Purpose: To investigate risk factors associated with generic *Eschericha coli* (gEc) contamination on fresh produce grown in certified organic farms using untreated manure.

Methods: A two-year (2017-2018) study was conducted in USDA-National Organic Program (NOP) certified organic farms (n=527) in California, Maine, Minnesota, and Maryland. Samples (soil, fresh produce, and water) were collected during the two growing seasons and were analyzed for gEc populations by mini-Most Probable Number (MPN) assay and confirmed by PCR. Farm, environmental and soil related risk factors were assessed using logistic regression models.

Results: Overall, gEc prevalence among all produce samples was 22.4% (118/527). Maine had the highest gEc prevalence (33.1% (41/124)), while California had the lowest (10.3% (23/223)). Soil moisture (OR=2.96, *P*=0.002) and precipitation on the sampling day (OR=1.01, *P*=0.020) significantly increased the odds of gEc produce contamination. Presence of foodborne pathogens in soil over 120 days after manure application (OR=5.14, *P*=0.009) increased the odds of produce contamination, while foodborne pathogens-positive soil under 120 days after manure application (OR=0.10, *P*<0.001) decreased the odds.

Significance: This study provides science-based information to identify potential risk factors influencing fecal bacteria contamination in fresh produce grown in soils amended with untreated manure on NOP-certified organic production systems.

T13-12 Contribution of Wild Bird Feces to *Salmonella* on Produce Plants

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❖ ***Developing Scientist Entrant***

Introduction: Identifying sources of foodborne pathogen transmission to fresh produce is crucial to prevent outbreaks.

Purpose: Wild birds have been linked to produce-related outbreaks, but basal prevalence and vector species have yet to be defined in the Southeast United States, complicating the ability of growers to manage pathogen risk.

Methods: To fill this knowledge gap, a total of 773 fecal samples were collected from 45 farms, ranging from small operations (<0.5 acres) to large commercial fields (>100 acres), across the Southeast US from May to October 2021 and 2022. In addition to fecal sample collection, Physical bird counts were conducted during each site visit to link observed bird communities to landscape and livestock factors that might increase risk of *Salmonella* incidence. Crops sampled included peppers, tomatoes, squash, cucumbers, eggplants, okra, and other above-ground produce.

Results: While *InvA* PCR results detected *Salmonella* in 9.75% of fecal samples, incidence of viable *Salmonella* identified by culture was significantly lower at 2.07%. Dried fecal samples were less likely to yield viable culture (chi-squared test, *P*<0.01). Additionally, no incidence of pathogen contamination from feces to produce on the sampled plant or to neighboring plants downwind was found. Sequence analysis of the *COI* gene from 75 fecal samples identified 19 different bird species, with five species linked to presence of viable *Salmonella*. An NMDS plot showed that bird species identified from feces found in produce fields were associated with a reduction in natural landscape.

Significance: While these results indicate some wild bird species pose a food contamination risk, overall incidence of *Salmonella* in wild bird feces appears low. The information from this study will be used to develop relevant on-farm bird deterrents to improve food safety in fresh produce.

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T14-01 Using Social Media to Reach Producers and Consumers of Microgreens: A Case Study

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Introduction: Microgreens are an emerging salad crop, which calls for customized education in food safety for both growers and consumers.

Purpose: An interdisciplinary team collaborated to design an information campaign in ways that appeal to growers and consumers. An initial user needs analysis showed consumers (n=680) commonly get information about microgreens from social media (49%) and/or cooking shows (49%). They most often eat microgreens at home (54%) and buy microgreens as a "living tray" (56%).

Methods: The team of microbiologists, digital media specialists, and instructional designers engaged in the *Transformational Design Process* to refine messages for an educational campaign for consumers and one for growers.

Results: Based on the needs analysis, the team created social media posts and animations to address key concepts. For example, 49% of the consumers in the needs analysis didn't understand the difference between sprouts and microgreens (P<0.001). In response, a social media infographic explained the difference. This initial post had high engagement (481 interactions; 981 views) and spread on several microgreens groups. It inspired copycat posts by microgreens influencers. This shows that science-based surveys on food safety can lead to successful interventions which meet consumer needs. The microgreens consumer social media campaign has been released yielding 29 posts on key messages. A campaign for growers is in progress and includes 3 animations in progress. A final toolkit will include a website, infographics in social media format, social media posts, and short animated videos at all-about-microgreens.org.

Significance: This campaign focused on basic information about microgreens storage and washing, common misconceptions, and safe growing practice, and is suitable for dissemination by food safety educators through the channels identified in the user needs analysis. The package provides trustworthy, science-based content that educators can distribute to their audiences for food safety education.

T14-02 Itips: Interactive Tools to Improve the Practice of Food Safety for Processors

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Introduction: Processing facilities and commercial incubator kitchens have created opportunities for processors to produce value-added products. While these processors from diverse backgrounds know their product well, they often lack experience and resources in providing appropriate training and evaluation specific to the food safety risks in these facilities and with their employees.

Purpose: "Interactive Tools to Improve the Practice of Food Safety" (iTIPS) is a multi-state (IA, MA, NM) project developing an interactive virtual lab to reach food processors and educators with an effective food safety digital training.

Methods: The interactive training was designed in a research-based process, with an interdisciplinary team of food scientists, gaming experts and evaluators. The team conducted a survey and semi-structured interview with a cohort of food industry partners (n=7) to better understand processors' needs. Program content priorities and interactive gaming layout was developed through a design summit. Using research data, the team identified audience needs, articulated intended educational changes for users, and planned educational activities to foster this change. The team refined the module based on user testing data.

Results: The cohort pointed to "time for training" and "cost of training" as the major issues standing in the way of food safety training. The panel ranked "preventive controls for human food", "sanitation and record keeping" and "allergen training" as the most important training topics needed. Additionally, they highlighted the need for short trainings, less technical and more grounded hands-on examples, and that trainings be bilingual (English and Spanish).

Significance: Creating an interactive module that is accessible and freely available online in English and Spanish enables stronger access to training tools to under-served communities with limited resources to help improve food safety practices and regulatory compliance.

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T14-03 Effectively Incorporating New Platforms into Education and Outreach Initiatives for Produce Safety Stakeholders: Learnings from a Year-Long Venture into the Virtual Space

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Introduction: Food safety education, outreach, and extension activities have, since 2020, increasingly transitioned to virtual forums to increase the number of stakeholders interacted with at a given time. A major barrier to virtual programming is determining what types of content transfer and make impacts with the audience.

Purpose: To analyze virtual methods of content delivery for engagement, interest, and value with fresh produce stakeholders to enhance education efforts.

Methods: A social media outreach program was implemented to assess stakeholder response to identifying food safety experts, answering common questions, obtaining relevant food safety publications, and increasing awareness of research and recommendations for the produce industry. Primary social media posts ("tweets") were released at least once per day on Twitter five days per week for 1 year (n=274). Eleven 90-min webinars were presented at a rate of approximately one per month to address emerging produce safety issues relevant to the industry using a speaker presentation or panel, question-and-answer session, and post-webinar survey (n=11). Data were collected using Twitter and Zoom Analytic software to assess engagement (engagement rate; attendance rate), interest (likes; registration), and content value (retweets; survey response) using Kruskal-Wallis and Dunn tests in RStudio Version 4.2.2.

Results: Primary tweet content was divided into advertisements (13.9%. 38/274), education (34.7%, 95/274), personnel introductions (18.6%, 51/274), publications (19.3%, 53/274), research updates (12.4%, 34/274), and other (1.1%, 3/274). Engagement did not differ by tweet content (P=0.2075), but interest (P<0.0001) and value (P=0.0065) did. Viewers valued advertising content significantly more than publications (P=0.0063) and research updates (P=0.0125). Average webinar registration and attendance were 522 and 374, respectively, with an attendance rate >70%. Both engagement and value increased over time for webinars, but interest did not.

Significance: Sole evaluation of commonly used engagement rates or interest metrics cannot effectively identify the types of virtual content valued by produce industry stakeholders.

T14-04 Thinking Outside of the Recipe Box: Food Safety and Nutritional Information in UK and U.S. Meal Kits

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Introduction: Growing in popularity, meal-kits give consumers the opportunity to prepare home cooked meals by providing boxes of fresh, measured ingredients with step-by-step recipe cards. Currently, little is known about the food safety and nutritional information included in meal kit recipes.

Purpose: Determine the provision of food safety and nutritional information in UK and US meal kit recipes.

Methods: Cumulatively, 485 meal-kit recipe cards (UK=359; US=126) from 19 providers (UK=8; US=11) were obtained from consumers via social media. A Qualtrics tool was developed to review inclusion of food safety and nutritional information.

Results: Although all eight UK and 10 of the 11 US meal kit providers included some form of food-safety related information, the information was often inadequate to ensure domestic food safety. For example, of meal-kit boxes that included perishable ingredients; 51% of UK recipes specified the need for chilled storage, whereas only 12% of US recipes provided such information. Only one recipe (from UK) referred to recommended temperatures (≤5°C).

Advice on washing fruit and vegetables were positive, with 88% of UK and 82% of US recipes referring to the practice. Handwashing before starting food preparation was stated in 46% of UK recipes but none of the US recipes. UK recipes provided handwashing prompts after 47% of occasions requiring handwashing such as after preparing raw poultry, while in US handwashing was only referenced on 6% of recipes. Subjective guidance for cooking adequacy were found; with UK statements (n=1306) focusing on visual assessment of colour (35%) while US statements (n=236) referred to cooking duration (40%). Nutritional information was provided by six UK providers and seven US meal kit providers.

Significance: This study has identified differences in food safety information communication by US and UK meal kit providers. Although food safety information was more frequently included in UK recipes, there is a need to understand if, and how, consumers engage with such information when following meal kit recipes.

T14-05 Understanding Establishment Food Safety Systems When RTE Product Tests Positive for *Listeria monocytogenes*

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Introduction: The Food Safety and Inspection Service (FSIS) initiates targeted inspection activities when ready-to-eat (RTE) meat and poultry products sampled by FSIS test positive for *Listeria monocytogenes*.

Purpose: This project aims to improve RTE product safety by understanding how establishments implement food safety programs after RTE product tests positive for *L. monocytogenes* and determining whether attention is needed with respect to *Listeria* control measures or other establishment characteristics.

Methods: Establishment compliance with HACCP, Sanitation, and *Listeria* Rule regulations was evaluated among 146 establishments where RTE product sampled by FSIS tested positive for *L. monocytogenes* from 2016 thru 2021. Analyses accounted for 874,931 inspection tasks scheduled for this cohort from 2016 thru 2021; noncompliance was documented by 15,879 of these tasks. Logistic regression assessed whether establishment characteristics (e.g., *Listeria* control measures, HACCP processing categories) were associated with noncompliance in the 30 days after product tested positive for *L. monocytogenes* and other outcomes (e.g., operational status, multiple product positives). Univariable odds ratios (OR) and 95% confidence intervals (CI) were estimated for each characteristic.

Results: While not statistically significant at P<0.05, odds of stopping FSIS inspected operations were lower among establishments with at least one "Fully Cooked – Not Shelf Stable" HACCP plan (OR = 0.495, 95% CI: [0.156,1.573]) than establishments without one. Odds of additional product testing positive for *L. monocytogenes* was higher among establishments using antimicrobial agents or processes (Alternative 2 Choice 2) to control *Listeria* (OR = 1.35, 95% CI: [0.439,4.155]) than those using sanitation (Alternative 3) where corrective actions (e.g., intensified sanitation) may be more readily implemented. Notably, 15 (10.3%) of the 146 establishments no longer produce RTE product.

Significance: These results may facilitate industry compliance with food safety regulations and statutes by enabling FSIS to develop targeted guidance or outreach for establishments with increased likelihood of adverse outcomes (e.g., noncompliance, operational status, multiple product positives).

T14-06 *E.coli* O157 Outbreak – 18 Years on: Reducing Risk through a Sector Specific Knowledge-Transfer Programme Engaging Government, Industry and Knowledge Partners – Case Study Impact

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Introduction: In 2005, an *Escherichia coli* outbreak in South Wales hospitalized 31 people, one of whom died. In total, 157 cases were recorded, and the outbreak source was identified as cooked meat from a local meat processing plant. The commercial impact was estimated to be a 10% reduction of the meat-sector value in Wales in 12 months following the outbreak. During the past 15 years, a bespoke knowledge-transfer project based on a 'Triple-Helix-Model' (government, industry and knowledge partners) has been designed and implemented to improve food-safety practices within the sector.

Purpose: The aim of this project included development and implementation of an intervention approach to positively impact food safety within private sector partners, including those in the meat sector.

Methods: This knowledge-transfer project content was defined by industrial partners and related to technical food-safety requirements. Each project involved a partner company and a university-based food safety expert. Clear, technical KPIs were defined for each project and measured throughout project implementation. Impact was measured by the number of successful British-Retail-Consortium-Global-Standards and food safety accreditations attained, financial company impact in relation to retained/new sales, creation/retention of jobs and the number of new markets accessed.

Results: Knowledge-transfer projects over the past 15years include engagement with 197 companies, implementation of 615 knowledge-transfer projects and utilization of $16.05 million of European Union funding. Outputs related to company performance were measured and validated at $208million. Knowledge-transfer activity led to 339new jobs being created with 2403jobs retained as a result of 218 new markets accessed (due to food-safety accreditations). Support for food safety in product development led to the successful launch of >699 products.

Significance: Outcomes of project development/implementation include an evolving knowledge-transfer based intervention to supply food safety expertise to the food sector, collaboration between knowledge-based partners across the country, a reduced risk of a food poisoning outbreak in partner companies and a mechanism to deliver governmental strategies in relation to food safety and sector growth.

T14-07 Exploration of Food Safety Culture Maturity and its Relation to Organizational and Employee Characteristics

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❖ ***Developing Scientist Entrant***

Introduction: Explorations of food safety management systems (FSMS) are available, but the next step is quantitatively exploring food safety culture (FSC) in the food processing industry.

Purpose: This research aims to reveal trends in maturity of included FSC dimensions, subcultures that could be present within organizations and groupings of companies based on maturity.

Methods: Twenty food processing companies were recruited and subjected to a food safety culture diagnosis through a mixed-method assessment. Food safety culture maturity, as perceived by employees, was compared based on the number of gaps (i.e. underdeveloped dimensions). Next, it was explored whether organizational characteristics (e.g. company size) and employee characteristics (e.g. leaders vs. non-leaders) were associated with perceived food safety culture maturity (1410 full responses) through a two-step cluster analysis and statistical tests (Mann-Whitney U and Kruskal-Wallis tests).

Results: The FSMS and the human-organizational building block had a relatively high frequency of gaps. In contrast, the human-individual building block of food safety culture seemed more mature in the sample. Significant differences in perceptions (P<0.05) were discovered between groups, based on the following organizational characteristics: company size, belonging to a larger group, producing private label or premium brand, place in the chain, food safety training frequency, certificates, maturity of the control and assurance activities, and maturity of the human-organizational dimensions assessed in the applied management interview. Concerning individual characteristics, significant differences (P<0.05) in perceptions were discovered based on whether or not the respondent occupied a leadership position, is in daily direct contact with food products, seniority, time since food safety training and psychosocial wellbeing.

Significance: Results can be used to develop food safety culture improvement interventions within companies as these might focus on lower perceiving (sub)groups of employees or would want to counter the formation of subcultures and could support food authorities to target specific groups of compa-

Technical

nies, inspection-wise.

T14-08 The National Antimicrobial Resistance Monitoring System Extending Retail Food Surveillance to Hawaii

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Introduction: Antimicrobial resistance (AMR) is an issue of concern for food safety and public health. Retail foods are one of the routes that transfer AMR to humans. The FDA National Antimicrobial Resistance Monitoring System (NARMS) tracks the AMR trends in retail foods.

Purpose: To enhance the NARMS geographical and population representativeness by extending retail food surveillance to Hawaii.

Methods: Starting January 2020, fresh retail meat (chicken, turkey, beef, and pork) and raw seafood (shrimp, salmon, and tilapia) were collected monthly from randomly selected grocery stores in Hawaii. *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* were tested in meat samples using the NARMS Retail Meat Isolation Protocol, while *Vibrio*, *Enterococcus*, *Aeromonas*, and lactose fermenters were tested in seafood samples using the NARMS Seafood Isolation Protocol.

Results: Between 2020 and 2022, 936 retail meat (282 chicken, 282 turkey, 186 beef, and 186 pork) and 216 seafood (75 salmon, 66 shrimp, and 75 tilapia) samples were collected. *Salmonella* was recovered in 16.0% chicken, 4.6% turkey, 1.6% beef, and 0.5% pork of tested samples and *Campylobacter* was recovered in 11.3% chicken and 0% turkey of tested samples (not tested in beef and pork). The recovery rates of *E. coli* ranged from 19.1% to 65.8%, while that of *Enterococcus* ranged from 48.1% to 86.5% in tested meat samples. Regarding seafood samples, *Vibrio* spp. was recovered in 6.7% tilapia, 21.2% salmon, and 71.2% shrimp of tested samples. The recovery rates of *Enterococcus*, *Aeromonas*, and Lactose fermenter ranged 40.0-84.8%, 57.3-78.7%, and 69.4-84.1%, respectively.

Significance: The NARMS retail food surveillance in Hawaii tracks the trends of AMR in retail foods in Hawaii.

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T14-09 Design and Evaluation of a Portable Atmospheric Cold Plasma Jet to Inactivate Pathogens from Fruits and Vegetables

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❖ ***Developing Scientist Entrant***

Introduction: Nowadays, atmospheric cold plasma demonstrates promising results in foodborne pathogen disinfection and hence can be utilized for in-field use.

Purpose: The study is focused to design and estimate the potential of a mobile cold plasma device for pathogen inactivation from fruit and vegetable surfaces.

Methods: A dielectric barrier discharge type plasma jet was designed using a plasma power generator fed with atmospheric air at a flow rate of 5 lpm. The current and voltage were determined using Agilent 54641D mixed signal oscilloscope coupled with a current transformer. The formed plasma gas species were identified using a BLACK-Comet Spectrometer connected with a F600-UV-SR fiber optic cable. The Matheson-Kitagawa gas detector and precision tubes were utilized to measure ozone generated from the plasma jet. Methylene blue (MB) verification test was conducted treating 0.001% analytical grade MB solution with plasma jet and measured the absorbance using UV-Vis spectrophotometer. Freshly harvested strawberry samples (n=30) were inoculated with *Aspergillus flavus* on the surface and CFUs were quantified before and after treatment for the plasma device efficacy determination. The samples were subjected to colorimetric test for aesthetic color value measurement using the Hunter Labs colorimeter.

Results: The discharge current during the plasma generation was ranged from 0.15 mA to 10 mA producing ozone varied between 9 ppm to 280 ppm at the tip of the plasma. The optical emission spectroscopy showed wide range of reactive oxygen species including singlet oxygen, ozone, and OH* radical. The MB test resulted maximum discoloration of 94.7% due to high ozone production from the system. At least 3 log reduction CFUs of *A. flavus* is expected from the plasma treatment with no losses in aesthetic outlook of the strawberry fruit.

Significance: The current results demonstrate promising usefulness of a portable plasma device in providing food safety solutions for fruits and vegetable farmers and food industries.

T14-10 Maturing Food Safety Culture with *Nudging* in Food Manufacturing Environments in the UK

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Introduction: Increasing number of public guidelines and private standards emphasizes the importance of food safety culture, challenging food industry to drive evidence-based culture change. "Nudging" is effective in influencing people's behavior and prompting culture change, but it has not been studied in the context of food safety culture.

Purpose: This research study aims to improve food safety culture via nudging in a weekly change cycle using a validated machine learning tool.

Methods: Each person was nudged every day through answering one question on food safety in nine UK food manufacturing companies (13 sites) from June 2021 to September 2022, resulting in over 180,000 answers. Machine learning generated a weekly action report for each company, from which the companies picked one incremental action to execute every week. Culture maturity was calculated as the running average of all responses. ANOVA was conducted to determine the maturity of food safety culture by dimension by functional work area. Logistic regression was conducted to determine how culture of food safety changed through nudging and to identify key drivers of change.

Results: Using the GFSI food safety culture position paper, companies have varying strengths on the five dimensions. Heatmap analysis indicates that "Adaptability" is the least mature dimension and "People" the second least mature. "Values and Mission" is most mature dimension in the participating companies, although how and why food safety was prioritised during business-critical decision-making was not always shared with team members. In the course of 16 months, nudging leads to improvement in food safety culture in several of the companies.

Significance: This study contributes to the currently scarce empirical evidence on how culture of food safety is improved. It is also the first study to use nudging to improve food safety culture.

Technical

T14-11 Comparing the Effect of Electrical Potential and Hydrogen Peroxide on the Efficacy of Atmospheric Pressure Plasma Jet to Reduce Three Salmonella Serovars at Three Exposure Times

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* continues to be a major risk associated with raw poultry warranting the need to explore novel technologies such as atmospheric pressure plasma jet (APPJ).

Purpose: Optimization of atmospheric pressure plasma jet (APPJ) to eliminate three different serovars of *Salmonella* inoculated on raw poultry skin.

Methods: Fresh chicken skin coupons (1 sq. cm) (n=189) were cut from the breast skin and stored at 4ºC. The coupons were inoculated individually with *Salmonella* Enteritidis (SE), *Salmonella* Heidelberg (SH) and, *Salmonella* Typhimurium (ST) to obtain a target inoculum of 10^4 CFU/cm^2. After a 30 min attachment period at 4˚C, the inoculated coupons were treated for 0, 10 and 20 min with different combinations of helium (He), hydrogen peroxide 3% (H_2O_2) and APPJ using 5.20 and 5.75 kV potential. Post-treated samples were vortex for 30 s in cryovials with buffered peptone water (2 ml), serially diluted and then spread plated on XLT4 agar plates which were incubated for 24 h at 37˚C. Typical Salmonella colonies were counted and reported as log CFU/sq. cm. The experiment was repeated as three separate trials with three samples per treatment for each trial. Data was analyzed using ANOVA ($P<0.05$) with Tukey's HSD to determine significant differences between treatment means.

Results: Plasma jet generated using 5.20 kV and 5.75 kV had a <1 log and 0.5-2.46 log kill at 20 min exposure, respectively demonstrating the significance electrical potential to eliminate *Salmonella.* Application of H_2O_2 + plasma at 5.75 kV was highly efficacious in eliminating ~1.8 log CFU of ST and SH while it did not make a significant change for SE indicating serovar-level differences in APPJ's efficacy to eliminate *Salmonella* on chicken skin.

Significance: The AAPJ is potential food safety hurdle technology, but significant research is warranted to ensure its efficacy.

T14-12 Induction of Viable-but-Non-Culturable *Campylobacter jejuni* Under Different Food Processing Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Foods are frequently exposed to complex environmental conditions, providing stress to induce bacteria to enter the viable-but-non-culturable (VBNC) state. As a leading cause of human gastroenteritis worldwide, *Campylobacter* can be induced to the VBNC state under stress conditions. However, how food processing can induce *C. jejuni* to enter the VBNC state and the potential role of foods in inducing VBNC *C. jejuni* remain largely unknown.

Purpose: This study aimed to investigate the effect of food processing conditions and food products on the progress of VBNC *C. jejuni* formation.

Methods: Culturability and viability of *C. jejuni* were investigated under chlorine treatment (25 ppm), aerobic stress (atmospheric condition), and low temperature (4°C) conditions. In addition, the behaviors of *C. jejuni* in ultra-high-temperature (UHT) and pasteurized milk were also monitored during refrigerated storage. The number of culturable and viable *C. jejuni* in both pure bacterial culture and food matrices was separately determined by propidium monoazide (PMA)-quantitative PCR and the plating assay.

Results: *C. jejuni* lost its culturability but partially remained its viability (1-10%) once bacterial culture was mixed with chlorine solution. In comparison, >90% *C. jejuni* was induced to the VBNC state after 24 h and 20 days under aerobic and low temperature conditions, respectively. The viability of *C. jejuni* remained stable during induction in UHT (>90%) and pasteurized (>95%) milk. The number of culturable *C. jejuni* decreased quickly in pasteurized milk although culturable cells could still be detected in the end (day 21). In contrast, culturable *C. jejuni* slowly decreased and became undetectable after >42 days of induction in UHT milk.

Significance: This study provides a better understanding of the induction of VBNC *C. jejuni* in the agro-ecosystem and aids in the development of innovative mitigation strategies to reduce the health risks associated with this microbe.

T15-01 Accelerated Inactivation of *Clostridium sporogenes* and *Bacillus subtilis* by Ohmic Heating

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Introduction: Prior work shows that the electrical components of ohmic heating (OH) cause accelerated inactivation of bacterial spores. In this work we explored the effects of electric field strength on the inactivation of *Clostridium sporogenes* PA3679 (a surrogate of *Clostridium botulinum*), and *Bacillus subtilis* PS533 (wild-type) & PS578 (lacking Small Acid-Soluble Proteins: SASP) & PS2318 (lacking recA gene important in DNA repair).

Purpose: Studying Ohmic heating effects on bacterial spore inactivation.

Methods: In this study we allowed the temperature to rise linearly by applying a constant electric field, and once the samples reached the set temperature, they were immediately cooled i.e., with zero holding time. We conducted experiments with three field strengths (30, 40, and 50 V/cm), and three final temperature settings (95, 105, and 115ºC for *B. subtilis*, and 110, 120, and 130ºC for *C. sporogenes*) respectively.

Results: Our results show that field strength had a strong effect on the inactivation of both spores, for instance the reduction in *C. sporogenes* counts increased from 1.71±0.06 CFU/ml for 30 V/cm to 3.9±0.52 CFU/ml for 50 V/cm at 130ºC. Similarly, for *B. subtilis*, the inactivation increased from 1.72±0.04 to 4.94±0.28 CFU/ml at 115ºC. We compared the inactivation data of ohmic with that of conventional heating by matching the temperature histories and we found a significant difference between ohmic and conventional. Furthermore, results of tests with spores of *B. subtilis* that lacked SASPs suggest that SASPs are one of the targets of the electric field. Also, our results with recA mutant follows similar inactivation trend compared to wild type spore, suggesting that recA is not a target of OH.

Significance: These findings add to our understanding of the nonthermal effects of OH and highlight the potential of OH to be used as an efficient way to kill spores without significantly affecting product quality.

T15-02 Effect of Plasma Activated Nanobubble Water (PNBW) Treatments on *Klebsiella Aerogenes* Biofilm on the Inner Surfaces of Piping: Numerical Simulation and Experimental Validation

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Introduction: Plasma activated nanobubble water (PANW) is an environmentally friendly sanitizers that can impart substantial shear stress to food contact surfaces. It also processes strong antimicrobial capability due to their reactive species, such as ozone, peroxynitrite, and hydrogen peroxide.

Purpose: Investigate and validate the approach of using PANW as a novel sanitizer to clean/sanitizer food contact surfaces with biofilm.

Methods: PAMW and PAW were generated by submerging dielectric barrier discharge electrodes in nanobubble water (NBW) and tap water, respectively. PANW, MBW, and chlorinated water (100 ppm) were used to clean the inner surface of PVC tubing inoculated with *Klebsiella aerogenes* at flow velocities between 0.1 m/s and 1 m/s. The number of *Klebsiella aerogenes* that attached to the inner surface of tubing after the cleaning were enumerated on a Tryptic soy agar plate. COMSOL Multiphysics was used to numerically simulation the nanobubble flow and the resulting shear stress that imparted to the bacteria was calculated.

Technical

Results: The plate enumerations show that PANW has the most potent antimicrobial effects, which reduced the number of bacteria on the inner tubing by 3.1 log CFU/cm at flow velocity of 1 m/s. With higher flow velocity, fewer bacteria were found on the inner surface of the tubing after cleaning. PAW and chlorinate water reduced similar number of bacteria (2.4 log CFU/cm to 2.6 log CFU/cm) at all the selected flow velocities. MBW only reduced the number of *Klebsiella aerogenes* by 0.7 log CFU/cm at 1 m/s flow velocity. The numerical simulations of the nanobubble flows shown that the presence of nanobubble has significantly increased the magnitude of shear stress imparted to the bacterial cells.

Significance: This study offers the food processors a non-thermal and environmentally friendly approach to sanitize the food contact surfaces without intensively using chemical sanitizers, which ensures the safety of foods with minimized chemical hazards.

T15-03 Frontiers in Application of Elevated Hydrostatic Pressure for Inactivation of Bacterial Pathogens and Endospores: Efficacy Augmentation by Mild Heat and Plant-Based Antimicrobials

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Introduction: Vast majority of treatments in private manufacturing currently utilize pressure intensity of 650 MPa and this pressure intensity alone is generally believed to be efficacious only against planktonic cells, thus unable to eliminate the bacterial endospores.

Purpose: Our studies investigated the use of 300 to 650 MPa pressure treatments augmented with mild heat (up to 60 °C) and plant-based antimicrobials for up to 5 and 3 log CFU/ml inactivation of pathogenic planktonic bacteria and bacterial endospore, respectively.

Methods: Studies presented are randomized complete block designs with two biologically independent repetitions as blocking factors. The microbial challenge studies were conducted using planktonic cells and endospores in liquid foods and ground meat with monitoring of temperature and pH values. The log-transformed microbial counts were statistically analyzed using Tukey-adjusted ANOVA at 5% type I error.

Results: Application of moderate pressure (<500 MPa) coupled with use of plant-based antimicrobials such as carvacrol, caprylic acid, and malic acid led to reductions of *Salmonella* serovars, *Listeria monocytogenes*, and Shiga toxin-producing *E. coli* at a rate comparable ($P \geq 0.05$) with 650 MPa-treatments. Additionally, endospores of four indicator microorganisms (*Alicyclobacillus acidoterrestris, Bacillus amyloliquefaciens, Bacillus atrophaeus, Geobacillus stearothermophilus*) were reduced ($P<0.05$) by up to >3 logs CFU/mL due to synergism of mild-heat, plan-based antimicrobials, and moderate pressure.

Significance: Current presentation illustrates that with proper validation studies, moderate pressure treatments of 300 to 500 MPa, augmented with mild heat and/or plant-based antimicrobials could ensure the microbial safety and shelf-stability of products to a degree comparable to those treated at elevated pressure of 650 MPa. This could lead to further retention of micronutrients, secondary metabolites, and organoleptic characteristics of pressure-treated products. Additionally, use of milder elevated hydrostatic pressure treatments could lead to cost-optimization, improving competitiveness of these products, and introducing this technology to commodities that are unable to withstand higher pressure-based treatments.

T15-04 Mechanical Abrasion is a Promising Non-Thermal Method for the Inactivation of *Bacillus* Endospores

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Introduction: Non-thermal processing methods such as high-pressure processing (HPP) are unable to produce shelf-stable beverages as they cannot kill bacterial endospores. Mechanical abrasion, which involves the agitation of spore suspensions in the presence of silica beads, has the potential to disrupt the spore structure and bring about their inactivation non-thermally.

Purpose: The study aimed to investigate the efficacy of mechanical abrasion in inactivating highly baro-resistant *Bacillus amyloliquefaciens* spores.

Methods: Spore suspensions of concentration 7.5 ± 0.6 log CFU/ml in 0.1% peptone water were prepared by heat shock (80°C for 10 min) and subjected to 2, 4 and 6 min of mechanical abrasion in a bead-miller surrounded by an ice jacket. Abrasion was delivered through 12 pulses, each lasting 30 s, with a 2 min relaxation after each pulse. In another scenario, the abrasion was delivered through two pulses of 5 min each, with 10 min relaxation in between. Surviving spores were serially diluted, pour-plated on nutrient agar and incubated at 37°C for 24 h. The Student's t-test was conducted to determine statistical significant differences between the population means before and after mechanical abrasion.

Results: With a 30 s pulse period, abrasion for 2, 4 and 6 min caused statistically significant ($P<0.05$) reductions of 1.6±0.3, 2.2 ± 0.8 and 2.3±0.7 log CFU/ml respectively relative to the initial population (7.5 ± 0.6 log CFU/ml). There were no significant differences ($P>0.05$) in the efficacy between the different abrasion durations. A pulse period of 5-min increased the reduction to 2.9 ± 0.7 and 3.8 ± 1.1 log CFU/ml after 5 and 10 min respectively.

Significance: With the potential to achieve greater reductions by optimizing the pulse width and pulse period, mechanical abrasion can be developed as a unit operation to achieve the non-thermal inactivation of spores and vegetative cells.

T15-05 A Large-Scale Investigation of Antibiotic-Resistance Genes and Associated Environmental Factors in Listeria Isolated from Natural Environments across the United States

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Introduction: *Listeria*, including *L. monocytogenes*, the etiological agent of listeriosis in humans, can rapidly acquire antibiotic resistance genes (ARGs), posing increasing food safety and public health concerns. It is believed that environmental changes may select particular antimicrobial-resistant clones that can then widely spread in nature.

Purpose: The purpose of this study is to advance our understanding of the distribution of ARGs in *Listeria* in the environment and underlying ecological mechanisms.

Methods: We leveraged a nationwide genomic and environmental dataset to characterize ARGs in 596 *Listeria* isolates detected in soil samples collected from nearly every state of the US. We further identified environmental factors that may promote its ARG acquisition using statistical tests.

Results: We found that the *lmo0919* gene, which confers lincomycin resistance, and *sul* gene, which confers sulfonamide resistance, are the most prevalent AMR genes (present in >80% isolates), followed by *fosX*, *norB*, and *lmo1695* genes, which confer fosfomycin, quinolone, and gallidermin/α-defensin resistance, respectively. *Listeria* isolates from the eastern US harbor significantly higher diversity and richness of ARGs compared to the remaining regions. The richness and diversity of ARGs in Listeria is significantly associated with soil property, climate, and land-use variables and can be accurately predicted by these variables using machine learning models (area under receiver operating characteristic curve of 0.85 and 0.81, respectively).

Significance: Collectively, our data suggest that the distribution of ARGs in Listeria in the natural environment is spatially heterogeneous, strongly shaped by environmental factors.

T15-06 Development of an Enzyme-Based Surrogate to Assess the Antimicrobial Effectiveness of Fresh Produce Washing

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Introduction: Adequate wash water disinfection is critical for preventing bacterial cross-contamination during fresh produce washing. Many approaches have been developed to process wash water, such as adding sanitizers and plasma treatment. However, antimicrobial effectiveness is complicated by various parameters including sanitizer concentrations and organic loads of wash water. To validate the antimicrobial effectiveness of produce washing, non-pathogenic surrogate bacteria are commonly used as indicators, but this requires time-consuming and labor-intensive microbiological testing.

Technical

Purpose: This study aimed to develop a non-living surrogate for rapid and visual assessment of antimicrobial effectiveness during fresh produce washing.

Methods: Catalase was selected as the enzyme-based surrogate because it is one of the key antioxidant enzymes that mitigate bacterial oxidative stress during sanitation. To quantify changes in catalase activity upon antimicrobial treatments, a visual assay based on foam height measurement was developed. Specifically, the reaction of catalase with hydrogen peroxide generated oxygen, which was trapped by surfactants to form a visible foam. Peroxide acid (PAA) and plasma-activated water (PAW) were used as representative sanitizers to assess the correlation between antimicrobial effects and catalase inactivation. All experiments were repeated for at least triplicates.

Results: A linear regression model was established between catalase concentrations and foam height (R^2=0.948). Despite significant differences in chemistries, both PAA and PAW inactivated catalase as a function of sanitizer concentrations. As a result, PAA and PAW dosages could be predicted by the foam height of catalase using one phase decay regression model. Besides, catalase inactivation with PAA and PAW showed an exponential growth regression to *Escherichia coli* reduction. This sensing approach was also applied to predict the antimicrobial effectiveness of PAA and PAW in simulated lettuce wash water to mimic fresh produce washing in packing houses.

Significance: This enzyme-based surrogate provides a fast and visual approach for assessing wash water sanitation during fresh produce washing.

T15-07 Cold Atmospheric Plasma to Control *Listeria* Strains and Extend Shelf Life of Fresh Blueberries (*Vaccinium corymbosum*)

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that represents high risk for consumers, since it can grow in refrigeration and can also develop acid tolerance response (ATR). Blueberries are hand-picked, packed and transported under refrigeration for up to 60 days without a microbial inactivation treatment. Cold atmospheric plasma (CAP) is a promising technology that can assure food safety and extend shelf-life.

Purpose: The aim was to study the survival of ATR (pH 6 and 5.5) *L. monocytogenes* and *L. innocua* in fresh highbush blueberries CAP treated at different times and shelf-life extension.

Methods: Fresh blueberries were spot inoculated with bacteria and then placed under a blowing arc CAP treatment system with a working distance of 7 cm and different treatment times (5, 15, 30 and 60s). Bacterial growth was evaluated using an automated spiral plating system using modified oxford agar. Shelf-life extension of uninoculated fruit samples was analyzed for visual appearance, yeast and molds counts, instrumental texture, surface color, pH, water activity (a_w), and soluble solids before treatment with CAP at time 0 and after 1, 4, 7 and 11 days of storage at 4°C and 90% humidity.

Results: CAP treatments over 30s demonstrated significant reductions in both pathogens under both ATR pH. Logarithmic reductions of 0.54 ufc/g were achieved after 60s CAP treatment on *L. monocytogenes* ATR pH 5.5. ATR pH 6 *L. monocytogenes* showed the least reduction with 0.28 ufc/g after 60s treatment. CAP 45s and 65s treatments showed significant reduction on yeast and molds at day 0, while no statistically significant effect on sample color, pH nor a_w was observed after 11 days. Visual appearance of fruit treated with 60s CAP showed loss of bloom by day 11.

Significance: CAP is a strong and safe antimicrobial treatment that is an alternative to prevent bacterial growth of fresh produce that cannot be washed.

T15-08 Not 'B*erry*' Fruitful: The Reduction of *Escherichia coli* on the Surface of Fresh Strawberry by UV-LED Technology is Limited by Complex Surface Structures

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❖ ***Developing Scientist Entrant***

Introduction: Ultraviolet-C (UV-C) light has potential as a dual-purpose technology for improving strawberry (*Fragaria* × *ananassa*) postharvest quality and food safety. Yet, as UV-C is not a highly penetrative form of irradiation, its antimicrobial efficacy is significantly reduced by shadowing from the fruits' surface structures (e.g., sepals, achenes).

Purpose: The purpose of this study was to determine if a table-top treatment chamber fitted with six, UV-Light Emitting Diodes (LEDs; 275nm) modules on all major sides could overcome this shadowing effect and provide simultaneous, high-powered UV-C treatment.

Methods: Strawberry fruits were dip-inoculated with rifampicin-resistant generic *Escherichia coli* to achieve an initial concentration of ~4 log CFU/g. To determine the contribution of the sepals to microbial survival, the leaves of select fruit were then either left intact or removed. The fruit were then treated individually on a 71.6% UV-transparent shelf at a dose of 0, 9.1, 18.2, or 27.3 mJ/cm^2 (0, 30, 60, or 90 seconds). Following treatment, the fruit were surface washed, and the surviving *E. coli* were enumerated on tryptic soy agar containing rifampicin (80μg/ml).

Results: There was a statistically significant effect of time (P<0.0001), with a highly suggestive effect of leaf presence (P=0.0568). Initially, strawberry fruit without sepals experienced a more rapid decline in microbial population. However, both groups reached a maximum log reduction after 90 seconds. A maximum log reduction of 1.48 (SD = 0.31) was observed in fruit with intact sepals, and 1.52 log (SD = 0.30) in fruit with no sepals. Non-linearity of the survival curves provided further evidence that the sepals were not majorly inhibiting UV-C efficacy.

Significance: The data improves the current understanding of UV-C limitations in the fresh strawberry industry. It is demonstrated that the strawberry sepals are not the major contributor to the shadowing effect, rather, the surface achene structures may pose the greatest challenge to UV-C technology.

T16-01 The Impact of Adoption of Milk Safety Practices on Food and Nutrition Security: Insights from Smallholder Dairy Farmers in Ethiopia

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Introduction: Food insecurity, and nutritional insecurity remain the prominent challenges in Ethiopia. To that end, good farming practices and productivity-enhancing agricultural technologies have been identified as pathways out of these problems, and are the priority agenda of the government policy in Ethiopia. Nonetheless, the impact of milk safety practices adoption on dairy farmers' food and nutrition security has not yet been addressed, especially at the smallholder farmers' level.

Purpose: The aim of this study was to evaluate the impact of adoption of milk safety practices on dairy farmers' food and nutrition security in Ethiopia.

Methods: A semi-structured questionnaire was employed to collect the primary data from 424 randomly selected dairy farmers in five districts in Ethiopia, of which 410 were used in analysis. A milk safety index was developed based on reported adoption of 45 recommended food milk measures. Different food types consumed by the households as well as expenditure on food purchased from the market were also collected, along with the sociodemographic, institutional and marketing variables. Generalized propensity score matching and a two-stage residual inclusion model were used to evaluate the impact of the adoption of milk safety practices on the households' food and nutrition security indicators.

Results: The adoption of milk safety practices had a moderate impact on food consumption expenditure (P<0.05) while it had no significant impact on food consumption score. In general, the impact of the adoption of milk safety practices on food and nutrition security of smallholder farmers is moderate in Ethiopia. This might be due to the high compliance costs of milk safety practices and lack of quality-based pricing systems.

Significance: The finding suggests that creating an enabling environment, which in turn, encourages adoption of milk safety practices would help improve the positive effects of the adoption of milk safety practices.

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T16-02 Interspecific Interactions Among Spoilage Bacteria of Dairy Origin in a Mixed-Species Model Biofilm

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Introduction: Diverse bacteria persist on food contact surfaces because of their intricate interactions in the form of mixed-species biofilms. Previously, we recovered *Stenotrophomonas rhizophila* (S1), *Bacillus licheniformis* (S2), and *Microbacterium lacticum* (S3) from a biofilm on the surface of a dairy pasteurizer after cleaning and disinfection. These species showed synergy in biofilm formation (2.5-fold increase in biofilm mass in co-culture). Only *M. lacticum* formed abundant biofilm in isolation (crystal-violet-screening in microtiter plates).

Purpose: The purpose of the study was to explore interspecific interactions and growth dynamics that led to net synergy in biofilm mass in the three-species biofilm model.

Methods: Bacterial biofilms in single as well as all possible dual-species combinations (S1+S2, S1+S3, and S2+S3) and a three-species combination (S1+S2+S3) were developed on stainless steel (AISI-304) coupons (28 x 10 mm) immersed in Brain-Heart-Infusion medium in 6-well microtiter plates. Individual cells in each combination were quantified using selective media plates. All species were used in equal initial proportion.

Results: *S. rhizophila* turned out to be the most dominant species in the three-species biofilm with a significant (P<0.05) increase in its growth from single-culture biofilm to three-species biofilm (6 to 8.3 log CFU/cm^2) followed by *B. licheniformis* (from 5.3 to 7 log CFU/cm^2), establishing commensalism and exploitative relationship, respectively, with *M. lacticum*. *M. lacticum* turned out to be the key species in promoting biofilm growth as it facilitated the growth of non-biofilm formers (S1 and S2) in the mixed community with a reduction in its own growth from 8 to 6.9 log CFU/cm^2 from single culture to three-species-biofilm, respectively. *S. rhizophila* stimulated matrix production in *B. licheniformis* without significantly affecting its growth which contributed towards the apparent increase in biofilm mass.

Significance: The knowledge on growth dynamics and key bacterial species in biofilms is important to control microbial biofilms of spoilage organisms on food contact surfaces.

T16-03 Does Desiccation Enhance UV-C Tolerance of *Cronobacter* spp.?

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Introduction: *Cronobacter* spp. is highly tolerant to desiccation and are capable of surviving in very dry environments and thus poses a significant food safety challenge in dried and desiccated foods. UV-C has been used to inactivate pathogens in air and on high contact surfaces. However, fluences required to inactivate desiccated *Cronobacter* spp. on surfaces is not known.

Purpose: Determine the fluences required for incremental inactivation of *Cronobacter* spp. desiccated on polystyrene surfaces.

Methods: Five dairy isolates belonging to three different species, *C. sakazakii* (CS1212, CS1214, CS1216), *C. malomaticus* (CM1246), and *C. turicensis* (CD1217) were individually desiccated on polystyrene surfaces and treated with UV-C doses of 0 to 30 mJ/cm^2 using a collimated beam device emitting UV-C at 253.7 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 identified using the plate count method and plotted against UV-C dose. The UV-C dose required for incremental inactivation for each isolate (non-desiccated and desiccated) was determined using linear and nonlinear regression. All results were analyzed using ANOVA, followed by a Tukey's test at a 95% confidence level to determine significant differences in log reductions between non-desiccated and desiccated cells for each UV-C dose and strain.

Results: UV-C doses required to inactivate non-desiccated and desiccated cells of *Cronobacter* spp. were not significantly different. However, doses required to inactivate *Cronobacter* spp. on surface were three time higher than those required to achieve similar inactivation of cells in suspension. For instance, 8.55 mJ/cm^2 was required to achieve a 4-log inactivation of CS1214 cells in suspension, while doses >30 mJ/cm^2 was required to achieve similar inactivation of cells on surface.

Significance: Desiccation does not enhance UV-tolerance of *Cronobacter* spp. Significantly higher UV-C doses are required to inactivate *Cronobacter* spp. on surfaces

T16-04 Microbial Control of Raw Skim Milk by Germicidal Ultraviolet Light (UV-C) Irradiation

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Introduction: Prolonged storage of raw milk in chilling stations before pasteurization increases the microbial load and causes the spoilage of milk in developing countries. The initial milk quality plays an important role in determining the end use of the milk and final quality of the milk product.

Purpose: In this proof-of-concept study, the efficacy of UV-C irradiation to control total number of aerobic bacteria (SPC) in raw skim milk (RSM) was investigated.

Methods: Optical properties of the RSM was measured using a double beam spectrophotometer connected to a single integrating sphere. Reduced scattering and absorption coefficients were mathematically calculated and accounted in the UV-C dose calculations. Fluid mixing was assessed using computational fluid dynamics. The absorption and reduced scattering coefficients were calculated as 12.58 ±0.8 cm^{-1} and 15.77 ± 1.04 cm^{-1}. 3ml (9 mm fluid thickness) of RSM 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, 20, 40 mJ·cm^{-2}) were delivered to the samples in triplicates followed by double platting and enumeration.

Results: Total number of aerobic bacteria in RSM was approximately 3.5 $\log_{10}$ cfu.ml^{-1}. 20 mJ.cm^{-2} of UV exposure reduced the total aerobic counts by 1.9 $\log_{10}$ cycles. At the highest dose of 40mJ·cm^{-2}, our results showed that UV-C irradiation completely inactivated total number of aerobic bacteria in RSM. This study demonstrated that initial microflora in RSM can be reduced or eliminated using UV-C technology.

Significance: UV-C technology can effectively control the microbial growth in milk at chilling stations, reducing spoilage. This will further improve the final microbial quality of pasteurized milk in developing countries.

T16-05 Validating Temperature for Growth, Nutrient Media, and Incubation Days for *Propionibacterium freudenreichii freudenreichii*, a Dairy-Originated Probiotic Bacterium, for *In Vivo* Studies

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Introduction: *Propionibacterium freudenreichii* is a dairy-originated probiotic bacterium capable of inhibiting the colonization of pathogenic bacteria in the host intestinal tracts

Purpose: Previous studies from our group suggest that *P. freudenreichii* has a beneficial effect on gut health. It can also be an alternative to antibiotics in poultry antagonizing pathogens. Adoption of *P. freudenreichii* for these purposes warrant testing of ambient conditions for its prolific growth outside the host

Methods: The growth of *P. freudenreichii* for seven days was assessed in two different nutrient growth media, deMan, Rogosa, Sharpe (MRS) and coculture media [MRS and Tryptic soy broth (TSB) at 1:1 ratio], at the temperatures suitable for bacterial growth (37°C) and poultry body temperature (42°C). *P. freudenreichii* inoculated in tubes containing 10 ml MRS or coculture media were incubated at either 37°C or 42°C, followed by an enumeration of bacterial

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growth on days 1 through 7. Three replicates were included for each treatment within a study, and the study was repeated. Data were analyzed using ANOVA. $P<0.05$ was considered significant.

Results: *P. freudenreichii* grew to >8 log CFU/ml at 37°C in MRS and maintained growth at the same rate over seven days. The probiotic grew in the coculture media in the range of 7.1 to 7.8 log CFU/ml. At 42°C, *P. freudenreichii* increased significantly (> 7.5 log CFU/ml) in MRS and had a reduced growth rate in the coculture media (6.5 log CFU/ml; $P<0.05$). Results indicate the use of MRS media for ambient growth of *P. freudenreichii* at 37°C for *in vivo* studies.

Significance: Validating protocols for consistent growth of *P. freudenreichii* for dosing studies in host animals, including poultry, is necessary before conducting expensive challenge models. The information will be used to develop safe and sustainable interventions against *Salmonella* Infantis and other problematic serotypes in poultry (USDA #2020-67017-30787)

T16-06 Prevalence and Antimicrobial Susceptibility Profile of *S. aureus* Isolates from Milk Samples Taken from a Texas Panhandle Dairy

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Introduction: *Staphylococcus aureus* is considered one of the most common mastitis-causing pathogens and has been found in outbreaks related to raw milk and other dairy products. Antibiotics are widely used within the dairy industry; thus, monitoring the prevalence and antimicrobial susceptibility of *S. aureus* is required for antimicrobial stewardship and is vital for a One Health approach.

Purpose: This study aimed to determine the prevalence and antimicrobial susceptibility profile of *S. aureus* isolates collected from milk samples at a Texas Panhandle dairy farm.

Methods: Quarter milk samples (n=323) were aseptically taken from a group of dairy cows previously diagnosed with mastitis within a Texas Panhandle-based dairy. Samples were plated on Accumast Plus plates, and *S. aureus* was isolated. All isolates were confirmed using MALDI-TOF MS (AXIMA-iD Plus). A subgroup of 95 *S. aureus* isolates was selected to conduct antimicrobial susceptibility testing, which was determined with a microbroth dilution assay, Sensititre™, using Mastitis CMV1AMAF plates. Clinical Laboratory Standard Institute Guidelines were used to interpret the results.

Results: Out of the milk samples tested, 62% had bacterial growth on the Accumast Plus plates (200/323). Of those bacterial isolates, 79% were positive for *S. aureus* (158/200). Most isolates (94%) showed co-resistance to penicillin and ampicillin (89/95). All isolates (95/95) were susceptible to pirlimycin, tetracycline, cephalothin, sulfadimethoxine, and oxacillin+2% NaCl. One isolate (1%) showed intermediate resistance to ceftiofur (1/95), while another (1%) showed intermediate resistance to erythromycin (1/95).

Significance: This study demonstrates that *S. aureus* had a high prevalence within the milk samples tested and, therefore, has the potential to end up in the food supply. While some resistance was seen, many antimicrobials had little to no resistance and could be evidence of good stewardship; however, it is still crucial to continue antimicrobial control efforts in animal production facilities.

T16-07 Inhibiting Potential of Selected Lactic Acid Bacteria Isolated from Costa Rican Agro-Industrial Waste Against *Salmonella* sp. in Yogurt

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Introduction: Lactic acid bacteria (LAB) isolated from agro-industrial waste may have bioprotective potential against foodborne pathogens in fermented foods.

Purpose: This research aimed to characterized LAB isolated from agro-industrial waste and explore its bioprotective potential against *Salmonella* in yogurt.

Methods: A total of 16 LAB isolates from Costa Rican agro-industrial waste, previously identified with 16S rRNA and with proven *in vitro* inhibitory capacity against *Salmonella,* were characterized according to auto-aggregation capacity, antibiotic resistance, presence of plasmids, and gelatinase and hemolytic activities. The bioprotective effect of the most promising strain, against a 4-strain cocktail of *Salmonella sp.* during yogurt fermentation, was also assessed. Yogurt pH and pathogen population were analyzed during fermentation until reaching a pH of 4.5. Experiments were performed in triplicate and data statistically analyzed.

Results: Three strains had good auto-aggregation ability and most strains were susceptible to all the antibiotics, except for vancomycin; five strains had no hemolytic activity and all were negative in the gelatinase assay and had no plasmids. *Lactobacillus pentosus* was added at 6 log CFU/g and did not significantly affect ($P>0.05$) the acidification curve of yogurt, in comparison to the control, and despite the significant bactericidal effect ($P<0.05$) against *Salmonella* during fermentation, the pathogen reduction was below 1 log CFU/g.

Significance: Emerging isolates of LAB may aid the dairy industry as bioprotective agents in fermented dairy products.

T16-08 Improvement Effect of Bioactive Compoundsderived from Bioconversion of Milk by *Lactobacillus Plantarum* with *Artemisia Herba-Alba* extract on Periodontal Inflammation and Diabetes

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Introduction: Many studies have reported that *Artemisia herba-alba* is effective on inflammation. However, the effect on periodontal inflammation still remains unexplored. Periodontal inflammation has been known to have a positive correlation with diabetes.

Purpose: The aim of this study was to investigate the improvement effect of periodontal inflammation and diabetes.

Methods: Milk was fermented by *L. plantarum* as a control (BM1), and *A. herba-alba* extracts were added to the milk followed by *L. plantarum* fermentation (BM2). Compounds of BM1 and BM2 were compared by HPLC analysis. Among them, compounds increased by the addition of *A. herba-alba* extracts were treated on murine macrophage-like cells (RAW264.7) stimulated by *Porphyromonas gingivalis*-LPS to investigate an alleviation effect of periodontal inflammation. After 12~24h, the amounts of nitric oxide (NO) and the expression levels of inflammatory cytokines were assessed. To examine their improvement effect on diabetes, same bioactive compounds as above were treated on human hepatocellular carcinoma cells (HepG2) whose insulin resistance was induced by palmitic acid for 24h. Lipid accumulation in HepG2 cells after being treated with the compounds was observed by Oil-Red O staining of the cells.

Results: As a result of HPLC analysis, proline and asparagine, which have been known to have anti-inflammatory effects, were significantly increased in BM2 compared to BM1 ($P<0.05$). The addition of proline and asparagine to RAW264.7 cells significantly decreased both the amount of NO and the expression levels of inflammatory cytokines compared to the control ($P<0.05$). Also, Oil-Red O staining showed that the compounds decreased lipid accumulation in HepG2 cells.

Significance: These findings suggested that proline and asparagine derived from BM2 can relieve periodontal inflammation and insulin resistance.

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Poster Abstracts

P1-01 ISO 16140-2:2016 Validation of Hygiena® Innovate Rapiscreen™ Dairy System as an Alternative Method for Commercial Sterility Testing in Nutraceutical Products

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Introduction: The nutraceuticals market is rapidly growing within the healthcare sector and from consumption by an increasingly health-conscious general public. Stringent sterility requirements for nutraceutical products consumed by high-risk patients puts more importance on microbial release testing, requiring commercial sterility testing with long incubation times.

Purpose: This study demonstrates that the Innovate RapiScreen™ Dairy system is suitable for commercial sterility testing in UHT Nutraceutical products using a validation guided by ISO16140-2:2016. The 30-minute method enables reduced time to results compared to standard plate-based commercial sterility testing.

Methods: Microbial cultures were prepared in Tryptic Soy (TSB) or Sabouraud Dextrose (SDB) broth with incubation at 37 °C for 24 hours. Overnight cultures were heat-shocked at 55 °C for 10 minutes before dilution to the desired spike level in maximum recovery diluent. Organisms were spiked into product packs (200-500ml) at Low (1-1.5 CFU/pack) and High (5-11 CFU/pack) levels. Spiked and uninoculated product packs were incubated for 14 days at 30 or 55 °C, depending on the tested organism. After incubation, aliquots were tested using Innovate and standard plate media (Tryptic Soy agar, Potato Dextrose agar, Orange Serum agar). Data from both methods was used to calculate the relative limit of detection (RLOD).

Results: Four organisms grew in the nutraceutical products: *Acetobacter aceti*, *Burkholderia cepacia*, *Lactobacillus brevis* and *Saccharomyces cerevisiae*. All four organisms were detectable using Innovate after 14 days incubation with an RLOD of $\leq$1 CFU per pack. Four other organisms used in preliminary studies showed no growth in the nutraceutical products: *Bacillus coagulans*, *Bacillus licheniformis*, *Geobacillus stearothermophilus* and *Lactococcus lactis*.

Significance: Although nutraceutical products are inhospitable to many microorganisms, those capable of growth (risking spoilage or infection) are detected by the Innovate RapiScreen method, decreasing the time to results in commercial sterility testing.

P1-02 Five-Log Reduction Times for Pathogenic *Escherichia coli* with Lactic and Acetic Acid Mixtures in a Model Vegetable Brine System

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Introduction: Antimicrobial weak acids are produced by heterolactic lactic acid bacteria during kimchi or sauerkraut fermentation; however, little is known about how mixed acids, pH, and temperature influence bacterial pathogen reduction.

Purpose: To estimate five-log reduction times (FLR) for *Escherichia coli* O157:H7 strains with mixtures of lactic and acetic acids in a model low-salt vegetable brine system.

Methods: Buffer models were used to estimate pH and protonated lactic and acetic concentrations in a non-inhibitory cucumber juice (CJ) medium. Mixed acid treatments (n=84) had selected combinations (0 to 50 mM, pH 3.2 to 4.2) for acid mixtures. Cocktails of four *E. coli* strains (two independent replications) at 10^8 CFU/ml were inoculated into 1.5 ml tubes at 10°C, 20°C, or 30˚C in a Peltier heating-cooling block under anaerobic conditions. Cells were plated for up to 10 days to determine CFU/ml. FLR and pH were analyzed by linear regression with JMP software.

Results: At 10°C there was a linear relationship between FLR and pH (R^2=0.93); however, at 20°C or 30°C, FLR values were non-linear with respect to pH and decreased as temperature increased. At 30°C all acid treatments had FLR of 4 days or less compared to 10 or more days for treatments at 10°C. The observed pH values for each acid combination in CJ showed an excellent fit to buffer model predictions, with a root mean square error of 0.04 pH units. All treatments with protonated lactic acid at 10 mM or greater had FLR values of 4 days or less, although some brines containing over 40 mM protonated acetic acid required 6 days or more at 10°C or 20°C.

Significance: Temperature and lactic acid concentration principally influenced FLR in a CJ model system. These data may help define worst-case scenario conditions to assure safe production practices for mixed acid fermentations.

P1-03 Combined Effects of Natural Glycolipids, Dimethyldicarbonate, and High-Pressure Processing on Microbial Spoilage of Orange Juice

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Introduction: High pressure processing (HPP) is a popular non-thermal processing method to preserve juice quality; however, the effects of HPP coupled with natural glycolipids (NG, Nagardo®) and dimethyl dicarbonate (DMDC, Velcorin®) (HPP+V+N) on juice microbial spoilage has not been investigated.

Purpose: The study aimed to compare the microbial spoilage of orange juice (OJ) treated with HPP and HPP+V+N to the spoilage of juices treated with pasteurization (P), and pasteurization coupled with same antimicrobials (P+V+N).

Methods: Pasteurized and unpasteurized OJ from the same batch were obtained from a local facility. Half of pasteurized OJ was supplemented with 30 ppm NG + 238 ppm DMDC (P+V+N). Unpasteurized OJ was subjected to HPP (600 MPa for 180 seconds at 5 °C) and HPP coupled with the 30 ppm NG + 238 ppm DMDC (HPP+V+N). The sampling frequencies for control (unprocessed), HPP and pasteurized samples were two days, two weeks, and three weeks, respectively. One mL of juice sample was serially diluted and spread on standard plate count (SPC) agar, acidified potato dextrose agar (APDA) for total yeast and mold count and 3M™ Petrifilm for lactic acid bacteria count. The study was performed using two biological replicates.

Results: The initial microbial load of the unprocessed OJ was 4.35±1.23 log CFU/mL. The unprocessed juice spoiled after 6 days, reaching 5.32±0.26 log CFU/mL. The shelf-life of pasteurized OJ varied widely, between 42 to 126 days. The shelf-life of P+V+N OJ was extended to a minimum of 126 days in both replicates. HPP extended the shelf-life of fresh OJ to 84 days (0.45±0.21 CFU/mL). NG and DMDC further extended the shelf-life to 126 days after which the microbial growth was below the detection limit (1 CFU/mL) in HPP+V+N group.

Significance: This study suggested that HPP coupled with glycolipids and dimethyl dicarbonate is a promising hurdle technology in extending juice microbiological quality.

Poster

P1-04 The Association of High Pressure Processing (HPP) Parameters and Products Characteristics with Safety Validation Study Outcome

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Introduction: High pressure processing (HPP) treated products are required to demonstrate a five-log reduction of the pertinent microorganism prior to commercialization. There are currently no standardized processing parameters despite the commercial success of HPP-treated food and beverage.

Purpose: The project aimed to study the associations between HPP processing parameters and product characteristics with respect to the validation outcome.

Methods: The Cornell HPP Validation Center performed validation studies to confirm if chosen HPP parameters would result in 5-log reductions of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes*. Generally, seven-log CFU/ml of 5-strain cocktails of each pathogen is inoculated in the food/beverage sample which gets processed by the specified pressure, dwell time and temperature. A product passes validation if the chosen HPP parameters result in at least five-log reduction of the pathogens and no growth over 1 log is observed after 50% beyond the expected shelf-life. Validation data of food products, along with category, pH, water activity and total soluble solids, from 2017-2021 were anonymized and used to build a generalized linear model (GLM).

Results: A total of 11 (out of 143) products failed validation for at least one of pathogens, with a mean pH of 5.29, which is 1.84 standard deviation higher than the pH across all products. Exploratory results from GLM showed that product category (single fruit juice, blended juices, tea, smoothies, hummus, dips, etc.) is not significantly associated with the validation outcome, suggesting that generalized HPP parameters (minimum 75,000 psi, 60-120 s, 5ºC) might be sufficient for acidified foods. pH was shown as the most significant parameter in determining the validation outcome. The coefficient for pH suggested that an increase of pH by 0.1 will reduce the validation success rate by a factor 0.48.

Significance: The study outcome can facilitate designing the processing parameters with given ingredients and physicochemical characteristics of food products.

P1-05 Promoting Probiotic Survival Under Harsh pH Conditions during Fresh Juice Storage by Microencapsulation

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Introduction: Microencapsulation is an efficient method that protects probiotics against external adverse conditions during storage of foods.

Purpose: This work studied the effectiveness of an encapsulation system to increase the survival of a probiotic strain under stress pH conditions during storage of fresh fruit juice.

Methods: *Lactiplantibacillus plantarum* 2035 (8 log CFU/ml) was encapsulated in a potato protein and pectin (PP-LMP) coacervate matrix. Then, the encapsulated cells were added in fruit juice to study their survival under harsh pH conditions (2.8, 3.0, 3.2, 3.4, 3.6) during storage at 10ºC (two batches with three replicates per batch). Free cells were used as controls.

Results: Results showed that the encapsulated cells exposed to pH 2.8 and 3.0 decreased gradually to reach 5.5 log CFU/mL after 27 days of storage, in contrast to free cells that had an immediate 2 log reduction and were not detected from the 12th day and onwards. At pH 3.2 and 3.4, both encapsulated and free cells declined by 2 and 4.5 log CFU/ml, to reach a final population of 6 and 3.5 log CFU/ml respectively after 27 days of storage. At pH 3.6, the encapsulated cells showed 1.5 log reduction early at storage (<5 days) and then maintained stable (6.5 log CFU/ml) while the free cells decreased gradually to reach below the detection limit (<1 log CFU/ml) at the end of storage. The protective effect of the coacervate was evident since the encapsulated cells were maintained in high levels (>5.5 log CFU/ml) in all cases of fruit juice storage.

Significance: It can be concluded that the PP-LPM coacervate matrix can be efficient in protecting the encapsulated probiotic cells under stress in a real food ecosystem.

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P1-06 Validation of Thermal Inactivation of *Enterococcus faecium* during Coffee Bean Roasting

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Introduction: Foodborne outbreaks and thermal tolerance of desiccated forms of foodborne pathogens have increased concerns regarding low moisture foods in the past decades. Coffee beans are associated with a food safety risk due to cultivation practices and post-process harvesting, which can potentially result in contamination.

Purpose: This purpose of this study was to validate the lethality of the coffee bean roasting process against *Enterococcus faecium* NRRL B-2354, a *Salmonella* surrogate, widely distributed in nature and known to have resistance to adverse environmental conditions.

Methods: Two types of coffee beans, Robusta and Columbian, were inoculated with *Enterococcus faecium* NRRL B-2354 at ~10^8 CFU/g. Inoculated beans (50 g and 100 g) were mixed with uninoculated beans at 1:9 ratio and portions of 500 g and 1000 g were used in the roasting process. The commercial scale roasting was conducted using a Probatino coffee roaster with an initial air temperature at 163°C and end temperature at 207°C with a total duration of 8 to 9 min. *E. faecium* count was determined by plating serial dilutions onto Trypticase Soy agar and incubated at 35°C for 24 h. The moisture, pH and water activity of the beans were measured before and after roasting. The samples were treated and tested in triplicates.

Results: In the untreated samples, the population of *E. faecium* was 7.8 log CFU/g in both bean types. For the roasted samples, there were >6 log CFU/g reduction of in all coffee bean samples. The roasting process significantly ($P < 0.05$) reduced the moisture and water activity level in the beans. The moisture of coffee beans dropped from ~10% to ~ 2.5% after roasting and the water activity decreased from ~ 0.60 to ~ 0.25 after roasting.

Significance: This study validated that commercial scale coffee roasting achieved five-log inactivation of *E. faecium* in coffee beans.

P1-07 Validation of a Kombucha Tea Recipe for Home Food Preservers

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***Introduction**:* The COVID-19 pandemic ignited increased consumer demand for home food preservation methods, underscoring a need for safe, validated recipes. The National Center for Home Food Preservation observed particular interest in fermented products, including kombucha.

Poster

***Purpose**:* Determine optimal sugar concentration for safety by monitoring kombucha pH, titratable acidity of acetic acid (TA), and surrogate pathogen lethality across the fermentation process.

***Methods**:* Tea samples were prepared with pre-manufactured SCOBY and sugar concentrations of 26 g/L, 53 g/L, or 80 g/L prior to inoculation with *Listeria innocua*, *Escherichia coli* K12, or an avirulent *Salmonella* strain. On Day 0, 7, and 14, 20 lL aliquots were collected to determine pH and TA. Additional 10 lL aliquots were collected, followed by serial dilution and plating on MOX (*Listeria innocua*), MacConkey (*E. coli)*, or XLD (*Salmonella*) selective media in duplicate. The treatment design was factorial between days and sugar concentration, and the experimental design was a randomized complete block with repeated measures and three biological replicates per treatment combination.

***Results**:* Mean log reductions from day 0 to 7 of 4.65±0.29 and 3.76±0.80 log CFU/lL in *Listeria innocua* and *E. coli* K12, respectively, were greater relative to corresponding reductions observed from Day 7 to 14 ($P<0.05$). Sugar concentrations of 26 g/L and 53 g/L demonstrated greatest lethality against *E. coli* K12 and *Salmonella* ($P<0.05$). No difference in lethality against *Listeria innocua* ($P\geq0.05$) was observed across sugar concentrations. Total average log reductions of 5.12±0.01, 4.44±0.92, and 5.35±0.85 log CFU/ml were observed for *Listeria innocua*, *E. coli* K12, and *Salmonella,* respectively. pH decreased from Day 0 to 14 for all treatments ($P<0.001$), with the lowest pH reaching 3.04±0.20 for *Salmonella*. TA increased from Day 0 to 14 ($P<0.001$) for all treatments.

***Significance**:* Microbial validation of recipes is crucial to allowing consumers to safely ferment food at home, preventing foodborne illness.

P1-08 Development of Cereals and Legumes Based Fermented Synbiotic Beverage

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Introduction: Fermented products are one of the ancient and commonly used plant-based health supplements for thousands of years throughout history.

Purpose: To develop a nutritious cereals and legumes based fermented synbiotic beverage.

Methods: The experimental plan was arranged on three levels that included the optimization of cereals and legumes based medium able to sustain bacterial growth based on sensory profiles, inoculated with mixed strains *Lactobacillus plantarum* and *L. caesi* and supplemented with inulin or oligofructose according, which was evaluation of survival growth of probiotic bacteria and physico-chemical parameters along with optimization the sensory characteristics of the mentioned product using adequate flavoring and stabilizers. All experiments were performed in triplicate were carried out and the results presented as a mean of the three values.

Results: In this research, the physicochemical analysis was done before and after fermentation; there were effective changes observed in the titratable acidity after fermentation by adding synbiotic mixture. Meanwhile, the titratable acidity value of the fresh sample was 19.8% and after fermentation increased in its value, was observed as 45%. Cereals-legumes based fermented synbiotic beverage was developed to evaluate the combined benefits of the probiotics *L. acidophilus*, *L. casei* and the prebiotic oligofructose. The survival growth of probiotic bacteria was observed by spread plate method and there was excess growth of probiotic that were too numerous to count. High viable counts are necessary to get the desired acid production and reduction in pH, which affects the products' organoleptic properties, its shelf-life, and prevents product contamination.

Significance: Legume and cereal based synbiotic beverage tend to have a higher shelf life, better nutritional composition, and accepted sensory quality. The blend of the cereal and legume in itself can be a rich source for the growth of probiotics.

P1-09 Change in Fermentation Conditions of Lacto-Fermented Sauerkraut Produced with Various Food Safety Process Parameters

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❖ ***Developing Scientist Entrant***

Introduction: Lacto-fermented foods such as sauerkraut have risen in marketing potential for local food processors, however, safety process parameters and established critical limits that validate process controls during production are limited.

Purpose: This project aims to investigate process parameters such as vessel type, salt concentration and starting pH that may affect the fermentation conditions of sauerkraut over time.

Methods: Sauerkraut was prepared with 4mm-shredded cabbage pieces and mixed with sea salt for 15 minutes, then filled in fermentation vessels with saltwater weight bags applied on top of cabbage below brine level and monitored up to fourteen days. This included observing changes in sea salt concentration (0.0, 1.6, 2.4, 3.2, and 6.4%), fermentation vessel type (8-ounce glass and plastic jars), and alternated starting pH using lactic acid powder (≥5.5, 5.0, 4.5, 4.0). The pH and lactic acid bacteria (LAB) counts were measured with statistical analysis ($P<0.05$, T-test) to investigate kinetic and microbial community change over time.

Results: Fermentation vessel type reported that both glass and plastic jars reach significantly below pH 4.6 by Day 5 (3.96 ± 0.08 and 4.15 ± 0.23 respectively), with less mold contamination observed in glass jar samples. Sauerkraut samples prepared with 1.6, 2.4, and 3.2% salt significantly lowered the pH by Day 5 (4.55 ± 0.12, 4.60 ± 0.11 and 4.45 ± 0.16 respectively) compared to the other concentrations. LAB counts significantly increased by 10 log for 1.6 and 2.4% salt sauerkraut after 36 hours of fermentation. Compared to the control (pH≥5.5), samples with starting pH 5.0 and 4.5 had a significant increase of LAB counts by Day 2 (four-log increase).

Significance: Identifying the safety parameters that contribute to producing microbial-safe lacto-fermented sauerkraut (in which the pH is reduced below 4.6 to monitor the pathogen growth threshold) can help provide process controls and subsequent technical support for food processors.

P1-10 The Out-of-Pack Challenge and Screening Testing of 5 Acidic Condiments Using a Panel of Spoilage Bacteria and Yeast on Innovate System

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Introduction: Condiment testing in-the-pack is a slow process due to low pH, high sugar, and salt concentrations. This study presents an out-of-pack method for the detection of spiked organisms in 24 hours using the Innovate System.

Purpose: To demonstrate the detection of spoilage bacteria and yeast using enrichment in 3 selective media: TSB, MRS and PDB.

Methods: 7 condiments–Mayonnaise, Hot Sauce 1 and 2, BBQ sauce, English Mustard, Dijon Mustard, and Thousand Island-were diluted into TSB, MRS and PDB at 1:10 dilution. Each of the product was tested in triplicate. Organisms were spiked and incubated as follows*: Bacillus cereus* and *E.coli* with product diluted with TSB *and* incubated at 35°C, *Lactobacillus fermentum* and *Lactobacillus brevis* tested with product diluted with MRS at 37°C, and *Saccharomyces cerevisiae* and *Zygosaccharomyces bailli tested with* product diluted with PDB at 30°C. Following incubation, the spiked products were tested on the Innovate System and plated on standard agar plates at 24h, 36h and 72h, respectively. The probability of detection was confirmed by comparing the positives on Innovate with corresponding growth of spiked bacteria on standard agar plates.

Results: In Mayonnaise, all organisms were detected at 24 hours except for *Zygosaccharomyces*. In Hot Sauce 1 and 2, *B. cereus* and *E. coli* were not detected, *L. fermentum* detected at 48 hours, *L. brevis* at 24 hours, *Saccharomyces* and *Zygosaccharomyces* at 24 hours. In BBQ sauce, all organisms were detected at 24 hours. In English Mustard, *B. cereus, E. coli* and *Zygosaccharomyces* were not detected, *L. fermentum* and *brevis* were detected at 24 hours. In Dijon Mustard, *B. cereus* and *E. coli* at 48 hours, *L. fermentum* and *brevis* at 24 hours, *Saccharomyces* and *Zygosaccharomyces* were not detected. In Thousand Island, all organisms were detected at 24 hours.

Significance: The rapid detection by an out-of-pack dilution in selective media followed by ATP measurements on the Innovate System will detect spoilage organisms more effectively in condiment products.

Poster

P1-11 Multistate Outbreak of Shiga Toxin-Producing *Escherichia coli* O121 Infections Linked to Frozen Falafel Consumption

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Introduction: In 2022, an outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O121 infections was identified; epidemiologic exposure evidence indicated frozen falafel was the likely source.

Purpose: To identify the potential source of and possible contributing factors to the contamination and prevent additional illnesses.

Methods: Illnesses were identified through PulseNet, and ill people were interviewed using standard food questionnaires. State and FDA laboratories used FDA BAM methods to recover *E. coli* O121 from frozen falafel and ingredient samples. State and federal officials collected records to determine the sources of ingredients and product processing. FDA's Coordinated Outbreak Response and Evaluation Network operated according to ICS principles and internal procedures to engage investigational partners.

Results: We identified 24 illnesses from 6 states: FL (2), IA (2), KS, MI (13), OH, WI (5). Clinical isolation dates ranged from July 24 to October 26, 2022. The manufacturing firm issued a recall on December 7, 2022, within 24 hours of learning about the associated illnesses. FDA collected 14 samples: three ingredients, including ground coriander, dill, and red pepper, and 11 finished product samples. State partners in Michigan and Ohio collected product samples from ill persons' homes. An open product sample collected by Michigan state partners yielded the outbreak strain by whole genome sequencing. The chickpeas used to manufacture the product were supplied as a raw agricultural ingredient. The manufacturer par-fried the product but did not sell it as ready-to-eat (RTE) and included cooking instructions to heat to 165F.

Significance: This outbreak is an example of a non-traditional product associated with STEC illnesses. Producers of non-RTE foods may wish to examine their processes and determine if there is opportunity to implement additional microbial control measures, even when products are labeled with clear cooking instructions. Opportunities exist to remind consumers to follow instructions provided on packages, even when products appear cooked.

P1-12 Prevalence and Genomic Characteristics of *Listeria monocytogenes* Isolated from Ice Cream and Associated Processing Environment in Hunan, China

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Introduction: A series of ice cream outbreaks associated with listeria contamination have been reported in recent years. Food processing environment can be the fundamental source of pathogen contamination. However, the incidence of *Listeria monocytogenes* in ice cream processing environments and the routes of pathogen transmission are not clear.

Purpose: This study aimed to investigate the prevalence of *L. monocytogenes* in ice cream processing plants and determine their genomic characteristics.

Methods: Two ice cream factories in Hunan were included for two consecutive years. Food samples (25 g), including milk powder, maltodextrin, sugar, fructose syrup, cream, production water, intermediate products, and finished products, were collected. Environmental samples were collected in the pre-treatment area, the filling area, and the product packaging area, including surfaces, equipment, and personnel. Collected samples were enriched for *L. monocytogenes* isolation and whole genome sequencing. Reads were analyzed in silico regarding their antibiotic resistance potential, virulence potential, and phylogenetic distances.

Results: Among the 353 collected samples, *L. monocytogenes* was detected in 2 out of 21 (9.52%) food samples and was prevalent across the processing environment (pre-treatment area: 7.55%, filling area: 18.05%, product packaging area: 8.6%). Virulence genes (e.g., prfA, plcA, hly, mpl, actA, plcB, inlA, inlB, inlC, inlE, inlF, inlH, inlJ, and inlK) were present in all isolates, and antibiotic-resistant genes (e.g., vancomycin) were also present in select isolates. Strains isolated from the same factory exhibited a closer phylogeny. Zero to two single nucleotide polymorphisms (SNPs) of *L. monocytogenes* genomes were found among samples of floors, worker shoes, and cartwheels, suggesting a close association between these samples. In the meanwhile, the high similarity between isolates in different years indicated potential *L. monocytogenes* persisters.

Significance: These data demonstrated that *L. monocytogenes* were prevalent in ice cream processing environments and had the potential to contaminate food and cause foodborne diseases.

P1-13 Investigation of *Salmonella* Prevalence and Quantification in Market Hog Lymph Nodes

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* contamination in market hog lymph nodes (LNs) can contaminate carcasses, as well as ground and comminuted pork meat products; therefore, a quali- and quantitative evaluation of LNs from several regions of the US can provide insight into *Salmonella* prevalence, concentration, and distribution during different seasons.

Purpose: This study was conducted to identify high risk LNs in market hog carcasses and investigate regional and seasonal variability of *Salmonella* in market hog LNs.

Methods: Market hog LNs were collected (N=4,132) from commercial pork abattoirs from 3 regions (east, central, and west), during 3 seasons (winter, spring, and fall). From 31~35 carcasses, mesenteric, subiliac, superficial inguinal, pre-scapular, axillary, and tracheobronchial LN, as well as tonsils were collected. Samples were trimmed, weighed, boiled, pulverized, and enriched with BAX® MP media. *Salmonella* was enumerated using BAX® System SalQuant® at 6 hours of incubation, and detected after 24 hours of enrichment. Prevalence and concentration data of mesenteric LN and tonsils were analyzed using the logit linear mixed model and lognormal accelerated failure time model, respectively; prevalence data of all other LN types were analyzed separately.

Results: Overall *Salmonella* prevalence was 36% for tonsils, 35% for mesenteric LN, and <10% for all other LNs. Season was significant for inguinal (P=0.008), subiliac (P=0.025), and pre-scapular (P=0.022) LNs, with winter prevalence (P<0.05) lower than spring (subiliac) or spring and fall (inguinal and pre-scapular). Region was significant for subiliac (P<0.001) and pre-scapular (P<0.001) LNs, with the eastern prevalence higher (P<0.05) than western and central for both LNs. Season-by-region interaction for *Salmonella* concentration was detected in mesenteric LN (P=0.014) and tonsils (P=0.031).

Significance: *Salmonella* prevalence varied by region and season for some market hog LNs, with tonsils and mesenteric LNs harboring the highest prevalence and largest concentrations. These data can be used by commercial pork abattoirs when assessing risk.

Poster

P1-14 Investigation into Online Reports of Adverse Reactions to Consuming a Ketogenic Meal Replacement Drink

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Introduction: Traditional foodborne outbreak investigations conducted by the US Food and Drug Administration (FDA) rely on cluster detection through laboratory surveillance systems and gathering of epidemiologic information. This provides highly accurate information but has lag times of several weeks. Some have proposed augmenting with information from novel online data streams such as social media to reduce lag times. Such novel data was used to investigate a meal replacement drink in 2022.

Purpose: Review FDA investigation, initiated in response to online food safety complaints, of Brand X ketogenic meal replacement drinks sold by Retailer A.

Methods: Data from FDA complaints, iwaspoisoned.com (IWP), and online reviews from Retailer A's website were retrieved for Brand X ketogenic drink, reviewed and self-reported symptoms summarized. Complaint volume was compared to Brand Y ketogenic drink, which had similar composition. Brand X product samples were collected, two from retail and one from a consumer's home; samples were screened for poisons/toxins, rancidity, medium chain triglyceride oil (MCT) levels and nutrient analysis.

Results: A total of 93 complaints were reviewed: FDA (6), IWP (34), online reviews (54); there was one known duplicate between IWP and FDA. Of these, 82% reported stomach/abdominal pain/cramps, 48% nausea, 23% vomiting, 24% diarrhea, 54% >1 symptom, 54% rapid onset (≤30 minutes). No etiologic agent was identified. For Brand Y ketogenic drinks, 0 complaints were found in IWP and 1 in FDA systems. Testing results were negative for toxins and rancidity; MCT and nutrient analysis were consistent with package labeling. Communication with Retailer A revealed Brand X keto drinks were already discontinued; after, Retailer A made product in stores unavailable for purchase. A dearth of clinical validation and product information posed challenges during the investigation.

Significance: Novel data sources may help bring food safety issues to light but pose challenges regarding quality and actionability.

P1-15 Characterizing Possible Disparities in the Incidence of Salmonellosis in the United States By Urbanicity and Community-Level Social Determinants of Health

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Introduction: Understanding differences in salmonellosis burden among different populations in the United States is useful for developing effective, equitable prevention programs.

Purpose: To identify potential disparities in salmonellosis incidence between counties with different SDOH profiles.

Methods: Using nationwide data from the Laboratory-based Enteric Disease Surveillance System from 1996 to 2018, we calculated age-adjusted county-level salmonellosis incidence (per 100,000 people) and linked county-level data from federal and non-profit sources on social determinants of health (SDOH; e.g., urbanicity, food environment [FEI]). Continuous variables were categorized into quintiles (Q), with Q1 representing counties experiencing the least and Q5 the most disadvantage. Differences in geometric mean (dGM) incidence between Q4 and Q2 were calculated. Incidence was stratified by SDOH alone and by SDOH and urbanicity. A positive difference indicated a possible disparity.

Results: Incidence was higher in large urban (GM=8.0; 95% confidence interval [CI]=7.6 to 8.5) and suburban counties (GM=7.3; CI=7.1 to 7.5) than rural counties (GM=3.3; CI=3.2 to 3.4). Counties with an index representing a high percentage of the population that identified as a racial or ethnic minority group or spoke English less than well had a higher incidence than those with a low percentage (dGM=2.1; CI=2.11 to 2.14). Differences were also observed among counties when stratified by FEI (dGM=0.6; CI=0.58 to 0.55), food insecurity (dGM=0.4; CI=0.43 to 0.45), and the percentage of the population without access to a car (dGM=1.5; CI=1.47 to 1.50). For some SDOH, disparities were only observed when incidence was also stratified by urbanicity.

Significance: Our analysis suggests that salmonellosis differentially affects persons in counties with populations that differ in SDOH profiles. These findings provide insights that can guide follow-on, quantitative studies, and develop population-specific prevention efforts.

P1-16 Relationship between Extreme Precipitation and Emergency Department Visits for Acute Gastrointestinal Illness in Toronto, Ontario, 2012-2022

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Introduction: Climate change is expected to increase the frequency and intensity of extreme precipitation events in Canada. These events can cause stormwater and sewage overflow events, flooding, and power outages, increasing population exposures to enteric pathogens from contaminated water and food.

Purpose: This study aimed to investigate the relationship between historical precipitation patterns and emergency department visits for acute gastrointestinal illness outcomes in Toronto over a 10-year period.

Methods: Distributed lag non-linear models were constructed with an overdispersed Poisson distribution. Extreme precipitation, defined as 95th percentile or higher mm of rainfall, was modelled as a 21-day lagged linear threshold. Models controlled for average temperature, day of the week, holidays, the COVID-19 pandemic period, season, the number of days with zero rainfall in the prior 30-day period, and time as confounding variables. A season-specific analysis was also conducted.

Results: Precipitation over the study timeframe ranged from 0 to 126 mm per day (median=0, 95th percentile=12.8, IQR=1.4). Gastrointestinal illness visits ranged from 12 to 180 per day (median=61, IQR=30). A 10-mm increase in precipitation above the 95th percentile had no significant relationship at lags of up to 15 days, and a slight negative relationship at lags of 17 to 20 days. However, when stratified by season, a significant positive relationship was found during the spring (Mar to May) at lags of 4 to 19 days, peaking at a lag of 11 days (incidence rate ratio [IRR] = 1.034, 95% CI: 1.013, 1.054). The overall cumulative effect across the 21-day lag period was also significant (IRR=1.760, 95% CI:1.318, 2.352). No clear relationships were identified for other seasons.

Significance: Extreme precipitation was associated with subsequent increases in emergency department visits in Toronto for acute gastrointestinal illness during the spring, suggesting possible enteric pathogen exposures through water, food, or other environmental pathways.

Poster

P1-17 Evaluation of Food Consumption Habits and Hygiene Practices in Consumers from Querétaro, Mexico, during the First Year of the COVID-19 Pandemic

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Introduction: During the pandemic, the security measures implemented led to a change in food consumer behavior which could have implications for Public Health.

Purpose: To evaluate food consumption habits and hygiene practices in food handling before and during the first year of the COVID-19 pandemic among consumers in Querétaro, Mexico

Methods: An online survey was conducted among the population of Querétaro, Mexico. The questionnaire was divided into four sections: 1) general characteristics (sex, age, education, and economic level); 2) consumption habits (home food consumption, street food consumption, food delivery, specific foods consumption frequency, etc.); 3)handling practices (hand-washing frequency, food packaging cleaning, and sanitizing frequency), and 4) health and food (suffering from gastrointestinal and chronic degenerative diseases).

Results: A total of 304 surveys were analyzed. The food consumption frequency of sweetened beverages, junk food, and alcoholic beverages increased during the first year of the pandemic, increasing from 18.4%, 13.2%, and 2.6% to 36.8%, 22.7%, and 10.2%, respectively, respectively. Before the COVID-19 pandemic, 54.3 % and 77.6 % always washed and disinfecting their hands before eating, while during the pandemic, the percentage increased to 77.6 % and 58.2%, respectively. Half of consumers mentioned that increase the consumption of unhealthy foods, while the other half of healthy foods. The 53.6% of the responders increased their weight, 19.1% developed a gastrointestinal disease, and 11.8 % a chronic degenerative disease.

Significance: Learning about the changes in food consumption habits and handling practices during the first year COVID-19 pandemic helped to understand its implications on public Health

P1-18 Wastewater-Based Epidemiology for Detection of Foodborne Disease

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Introduction: Current foodborne outbreak detection relies on sick people to seek medical help, delaying foodborne outbreak detection and case reporting.

Purpose: An investigation of wastewater-based epidemiology (WBE) for surveillance of six foodborne bacterial pathogens as a first step in the development of a new, active surveillance system.

Methods: Weekly wastewater samples were collected from the city of Guelph (Ontario, Canada) Wastewater Treatment Plant from June 2021-June 2022. Five hundred milliliters of each sample was pumped (average flow rate: 60 mL/min) through a disposable K-Cup paper filter that was housed in a 3D-printed cartridge. Next, DNA was extracted from the retentate using the DNEasy Blood and Tissue kit (Qiagen). Samples were analyzed by quantitative real-time polymerase chain reaction using SureTect RT-PCR Assays (ThermoFisher Scientific) for *Salmonella spp., Escherichia coli (*Shiga-toxin producing *E. coli* (STEC) and *E. coli* O157:H7*), Campylobacter spp., Vibrio vulnificus, Vibrio parahaemolyticus, and Listeria monocytogenes*. Concentration threshold (Ct) values below 40 were considered positive. Laboratory-confirmed enteric case data was obtained from the Wellington-Dufferin-Guelph Public Health Unit (WDGPHU) for analysis.

Results: Weekly wastewater samples yielded positive results with varying concentrations for *Salmonella spp.*, STEC, and *L. monocytogenes*, which were plotted using scatterplots. The scatterplots were overlayed with case data. For both STEC and *L. monocytogenes*, less than 5 cases were reported in Guelph over the study period, and no relationship between the wastewater signal and cases were observed. A peak in *Salmonella spp.* concentration in wastewater was observed between December 10, 2021 and January 31, 2022, which immediately preceded a rise in salmonellosis cases from January 2022 to March 2022. Based on this result, the limit of detection of WBE was determined to be between 1.5 and 2.75 cases/100,000 people for *Salmonella*.

Significance: WBE appears able to detect outbreaks of foodborne bacterial pathogens when sufficient numbers of people are infected.

P1-19 Wastewater-Based Epidemiology of *Providencia Rettgeri*

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Providencia rettgeri* is associated with traveller's diarrhea and has been isolated from meat samples in several Asian countries, suggesting meat as a potential vehicle for contamination. Wastewater-based epidemiology is an emerging approach for surveillance of both common and uncommon foodborne pathogens.

Purpose: To investigate the utility of wastewater-based epidemiology (WBE) for genomic surveillance of *Providencia rettgeri.*

Methods: Wastewater samples were collected from the city of Guelph (Ontario, Canada) wastewater treatment plant. Each sample (500 mL) was pumped (average flow rate: 60 mL/min) through a disposable K-Cup paper filter that was housed in a 3D-printed cartridge. The retentate was enriched in 5 mL tryptic soy broth and plated onto thiosulfate-citrate-bile salts-sucrose agar and incubated at 37 °C overnight. Several isolated colonies with typical morphology were picked and DNA was extracted using the DNEasy Blood and Tissue kit (Qiagen). DNA libraries were prepared using the Illumina DNA prep Tagmentation and IDT for Illumina DNA/RNA UD Indexes. Paired end (2 x 150 bp) sequencing was performed on the Illumina MiniSeq system. Trimmed reads were assembled *de novo* using Skesa v2.4.0. From the draft genomes, the mobilomes, (MOB-suite v3.1 and Virsorter2 v2.2.3), resistomes (CARD) and virulomes (VFDB) were determined. The phylogeny and the genetic relatedness between isolates and those in public database were inferred using SNIPPY v4.6, and IQtree.

Results: Five *P. rettgeri* isolates were sequenced. The genomes' size ranged from 4.17 mb to 4.35 mb. No antibiotic resistance, virulence genes or plasmids were observed in the genomes. A SNP-based phylogenetic tree of 184 *P. rettgeri* genomes (from NCBI) from 10 different animal hosts was constructed, and all *P. rettgeri* strains isolated from wastewater were nested within the same clade together with isolates recovered from human urine.

Significance: WBE is a useful approach for genomic surveillance of emerged and emerging foodborne pathogens.

P1-20 The Development of an Egg-Specific Targeted Mass Spectrometry Method: Target Peptide Refinement

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❖ ***Developing Scientist Entrant***

Introduction: The detection of egg residues in processed food products is a critical challenge for food allergen management. Parallel reaction monitoring (PRM) is a mass spectrometry (MS) method and can potentially detect and quantify low levels of egg protein in processed foods.

Purpose: This study aims to refine peptide targets that detect egg protein in four processed food matrices (sugar cookies, pie crust, pasta, and ice cream).

Methods: Protein was extracted from whole egg powder (WEP) and the four matrices (blank and incurred with 10,000 ppm WEP), followed by trypsin digestion and clean-up. Previous non-targeted MS work identified 88 WEP-specific candidate peptides in the four incurred matrices using standard discov-

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ery proteomics identification software (PEAKS Studio v8.5). A targeted PRM-MS method was generated with a scheduled inclusion list. Candidate peptides were screened in serially diluted food and WEP samples (n=4) (equivalent to 250, 500, 750, and 1000 ppm WEP). PRM results were analyzed in Skyline. A refinement process was conducted to select robust target peptides.

Results: The peptide selection criteria developed for application across all food matrices included the following performance metrics: correlation of relative product ion intensities to library spectra obtained from non-targeted MS analysis (Skyline dotp value ≥ 0.75), signal quality (at least three product ions detected), peak shape (by manual evaluation of concurrent product ions) and peak area (sum of top 3 product ions). Based on targeted MS data, 11 WEP-specific peptides performing well against the criteria were selected for the four matrices (7 in cookies, 5 in pie crust, 7 in pasta, and 8 in ice cream). These peptides originate from egg white proteins (6 peptides), egg yolk proteins (4 peptides), and apovitellenin-1 (1 peptide).

Significance: This study identified 11 multi-food applicable WEP-specific peptides that can be used in a sensitive targeted MS detection method.

P1-21 Development and Evaluation of a Real-Time PCR Assay for the Detection of Bovine Milk in Foods

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Introduction: The Food Allergen Labeling and Consumer Protection Act lists milk as a major food allergen in the United States. This assay is designed and optimized 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 for the COI mitochondrial gene target for *Bos taurus* (domestic cow). Cross-reactivity was tested against several species including pork, chicken, bison, water buffalo, camel, goat, and sheep. Modifications to the primer and probe set were made to eliminate cross-reactivity, particularly with bison and water buffalo. PCR conditions were optimized, and bovine milk DNA was tested for detection and assay efficiency with known concentrations.

Results: Efficiency tests of the assay in buffer with beef and milk DNA have shown good linearity with R^2 values of 0.99. The range of detection for milk and beef DNA in buffer is 10 fg/µL to 1 ng/µL. LOD is approximately 10 fg for both milk and beef DNA. Based on previous studies, LOD, range and efficiency are expected to be maintained when spiked into complex food matrices. There is no cross-reactivity with other common species that produce commercial dairy products.

Significance: While ELISA kits are the most commonly used detection method for allergens, they typically cannot distinguish between closely related species of the allergen. This work validates a method to detect and distinguish bovine milk in commercial food products and also demonstrates the specificity and sensitivity of the assay given its ability to detect small quantities of total DNA in milk products.

P1-22 Are Vegan Products Safe for Consumers Allergic to Eggs and/or Milk?

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❖ ***Developing Scientist Entrant***

Introduction: Allergic consumers use ingredients lists and food labels to inform their purchasing decisions and avoid allergens. The availability of vegan food products is on the rise and may be perceived as a safe alternative by consumers allergic to animal proteins.

Purpose: This research aims to determine if vegan and other food labels indicating the absence of animal-derived ingredients are suitable safety indicators for egg and milk-allergic consumers.

Methods: A total of 128 products of 85 brands with “vegan”, “plant-based”, “dairy-free” and/or “egg-free” labels were purchased from 3 retailers in Quebec, Canada. A minimum of three samples were selected from each of the main food categories that included the targeted labels (i.e., baked goods; meat, egg, and dairy alternatives; crackers; cookies; sauces; dark chocolates; soups; and ready-to-eat meals). Among these, products with precautionary allergen labelling for egg and/or milk were purposely selected. One-gram homogenized samples were analyzed to quantify milk (n=92) and egg (n=68) protein concentrations with sandwich-type ELISA kits (RIDASCREEN FAST Milk and RIDASCREEN Fast Ei/Egg protein, R-Biopharm) in duplicates. Samples with concentrations greater than the limits of quantification (LOQ = 2.5 ppm for milk protein and 0.5 ppm for whole egg powder) were considered positive.

Results: Milk proteins were not detected in 95% (87/92) of the samples tested. Samples where milk proteins were detected included dark chocolates (n=4; > 67 ppm, 1 brand) and baked goods (n=1; 2.59 ppm, 1 brand). Egg proteins were not detected in any (68/68) of the samples analyzed.

Significance: These results suggest that, under current manufacturing practices, vegan and similar labels may be used by egg and milk-allergic consumers in Quebec to guide prepackaged food purchasing decisions. However, establishing specific requirements for the use of these labels by food manufacturers would be needed to ensure the safety of consumers allergic to animal proteins.

P1-23 Determination of Indicative Levels for Precautionary Allergen Labeling (PAL)

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Introduction: Precautionary Allergen Labeling (PAL) is voluntarily used to address the unavoidable presence of allergens in food; however, there are no internationally agreed lower or upper action levels to determine ‘when’ and ‘when not’ to apply PAL.

Purpose: This project aimed to develop *indicative levels*; that is, the maximum allergen carry-over level in a product with a PAL for a given allergen to prevent serious reactions in consumers.

Methods: Carry-over levels were determined after a standard allergen cleaning in difficult-to-clean product categories at 12 chocolate plants and 8 biscuit plants across Europe. Worst-case scenario, and homogeneous and heterogeneous carry-over scenarios were evaluated. Product samples were collected at pre-determined time points to be analyzed using quantitative ELISA methods. The level to ensure consumer protection was established by comparison to threshold distribution curves, data modeling based on consumption of the 95th percentile, probabilistic modelling, literature revision and consumer surveys.

Results: The data for chocolate plants followed a consistent pattern with medium to high allergen levels detected in the first minutes that fell off sharply in a short time. Except for two out of the 12 chocolate sites, the levels detected at t_0 were generally less than 200mg/kg. The data generated from the biscuit sites showed a similar pattern of rapid decrease over time for all the allergens; however, levels were generally lower. Milligram doses for each allergen based on these data were compared to threshold distribution curves (mg protein) and to a probabilistic model that considered prevalence data as well as consumer avoidance data for products with PAL. Taking a conservative approach while considering the probabilistic modelling and the results from the plant studies, indicative levels for the individual allergens were set between ED_{05} and ED_{10}.

Significance: This approach gives a scientifically defensible basis to PAL and allows for a higher standard to protect allergic consumers.

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P1-24 Droplet Digital PCR for Detection of Allergenic Peanut in Food Ingredients

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Introduction: Detection of food allergens requires analytical methods which are highly sensitive and specific. PCR-based techniques have proven to be robust for detection of allergenic foods in a variety of matrices, and they do not suffer the same drawbacks as antibody-based detection methods.

Purpose: The purpose of this work was to evaluate droplet digital PCR (ddPCR) for detection of peanut, a major food allergen, in food ingredients and compare its performance to that of real time PCR.

Methods: Experiments were conducted using three food ingredients, chickpea flour, cocoa powder, and garlic powder, which are known to be challenging and have presented peanut contamination concerns in the past. Whole peanut was spiked into each ingredient at 0.1, 1, 10, 100, 1000, 10^4, and 10^5 mg peanut per kg food matrix (mg/kg, or ppm). Samples were homogenized and total DNA was extracted using a commercial plant DNA extraction kit. Real time and droplet digital PCR were conducted using an assay which targets three different peanut-specific regions in the chloroplast genome (*matK*, *rpl16*, and *trnH-psbA* spacer).

Results: Droplet digital PCR was more precise than real time PCR both between and within targets. ddPCR had a quantitative range of 1 to 10^4 ppm with relative standard deviations (RSD) less than 25% for all three peanut targets. Real time PCR had lower limits of quantitation at 10 to 100 ppm, depending on target, and a linear range up to 10^6 ppm. Both methods had lower limits of detection at 0.1 ppm.

Significance: This work has shown that the latest generation of PCR technology, droplet digital PCR, is effective for detection of allergenic foods at trace levels. It has greater precision than real time PCR and can therefore enhance the reliability of allergen detection.

P1-25 Polymerization-Mediated Amplification in a Sandwich Immunoassay for Protein Detection

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❖ ***Developing Scientist Entrant***

Introduction: Peanut allergy is one of the most prevalent, long-lasting, and severe food allergies caused by a type I hypersensitivity reaction to immune system.

Purpose: The goal of this research is to develop a biosensing-based, simple, robust, inexpensive, rapid, and sensitive peanut allergen detection platform to protect individuals with peanut allergy from accidental/unintentional exposure.

Methods: This platform uses real-time molecular growth via a *Polymerization-Mediated Amplification (PMA)* process to achieve label-free and detector-free allergenic protein detection. A polymerization reaction is activated upon a biomolecular binding that subsequently leads to the formation of polymer brushes tethered to a solid support. The polymerization reaction consequently amplifies the traditional sensing readout from one detection tag per biological binding event to having more than 100 reporting tags per binding event. The formed thin layer of polymer film is readily distinguishable by the naked eye as an opaque spot on the surface. The directly visible results eliminate the need for sophisticated readout equipment and, thus, enable field analysis outside of well-equipped laboratories.

Results: Four different designs for bovine serum albumin (BSA)-biotin-streptavidin binding model based on *Polymerization-Mediated Amplification (PMA)* have been successfully established as proof-of-concept for peanut allergenic protein detection. Direct visualization of 0.6 µg biotin was demonstrated in a detector-free fashion.

Significance: The work is fundamentally different from other food allergen detection methodologies. The universal amplification concept is also applicable to other sensing needs in agricultural and food systems.

P1-26 Effect of Storage Conditions on Occurrence of and Mycotoxin Production by Mycotoxigenic *Aspergillus* in Peanut

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Introduction: The safety and quality of peanuts are mostly affected by aflatoxins and ochratoxin A produced by the genus *Aspergillus*, which occurs during production process.

Purpose: Exploration of storage conditions (temperature and relative humidity) to prevent mycotoxigenic fungi and mycotoxin production in peanut.

Methods: Unshelled peanuts were stored at 15°C , 20°C , 25°C and 30°C with 95% RH for five months and the peanut samples were collected monthly. After removal of the shells, fungal occurrence and mycotoxins were analyzed. *Aspergillus* species and toxin chemotype were identified using molecular marker genes and mycotoxin biosynthesis genes, respectively. Aflatoxins and ochratoxin A were quantified using immune-affinity column (VICAM, MA, USA) and ultra-performance liquid chromatography.

Results: Frequency of *A. flavus* and aflatoxins levels increased with temperature and storage period. At 25°C and 30°C , *A. flavus* and aflatoxins levels increased after three months of storage. The highest frequency of *A. flavus* was detected at 25°C (27.3±17.2%) and the highest aflatoxins levels were detected at 30°C (311.8±95.9 µg/kg). At 15°C and 20°C , frequency of *A. flavus* increased to 16.7±13.9% after four months but aflatoxins levels were below the regulatory level (15 µg/kg). *A. niger* and *A. welwitschiae* were also detected with the highest frequency at 25°C (18.7±6.6%), whereas ochratoxin A levels were the highest at 30°C (32.2±43.6 µg/kg). Water activity of peanuts was 0.88 at 25°C and 0.87 at 30°C . These results showed that maximum growth of mycotoxigenic *Aspergillus* occurred at 25°C in peanuts, whereas maximum production of aflatoxins and ochratoxin A occurred at 30°C .

Significance: We suggest to store unshelled peanuts at below 20°C when RH is high (95%) to prevent from aflatoxins and ochratoxin A contamination by mycotoxigenic *Aspergillus*.

P1-27 Effect of Storage Conditions on Occurrence of *Fusarium oxysporum* and Its Mycotoxins in Ginger

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Introduction: Ginger is used worldwide as a spice and a herbal tea. Occurrence of mycotoxins such as aflatoxin, ochratoxin A, beauvericin and enniatins has been reported in ginger and ginger products.

Purpose: To investigate effects of storage conditions on occurrence of mycotoxigenic fungi and mycotoxins in ginger

Methods: Gingers from two different regions were stored at 5°C /73% RH (average), 14°C /68% RH or 13°C /96% RH for six months. Ginger samples were collected at the 0, 2, 4 and 6 months. Fungal occurrence was investigated using a direct plating method and representative fungal isolates were identified using molecular marker genes. Sixty-four *F. oxysporum* strains grown on potato dextrose agar and ginger samples were analyzed for mycotoxin using modified QuEChERS method for extraction and LC-MS/MS.

Results: Ginger samples (n=60) were mainly contaminated with the members of *Fusarium* (64.8±18.6%) throughout the storage period. *Aspergillus* and *Penicillium* were detected at the levels of 3.8±7.5% and 0.1±0.5% in average, respectively. *F. oxysporum* (53.9±20.2%) was predominant, whereas *A. flavus* was not detected. The frequency of *F. oxysporum* increased with storage time, but the occurrence and concentration of mycotoxins were significantly affected by storage conditions. At 5°C /73% RH, fungal frequency was the lowest, but concentrations of enniatins (sum of A, A_1, B and B_1) (2,029.981±1,748.144 µg/kg, mean±SD) was the highest. The highest incidence of *F. oxysporum* (71.9±9.8%) and the highest concentration of beauvericin (725.678±543.013 µg/kg) were observed at 14°C /68% RH. These results suggest that the most suitable condition for ginger storage was 13°C / 96% RH. All of *F. oxysporum* strains tested produced beauvericin (15.858 to 93.392 mg/kg) and three of them produced enniatin B (2.756 to 7.950 mg/kg) together.

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Significance: We suggest storage conditions for ginger to minimize contamination with mycotoxigenic fungi and mycotoxin. This is the first report on the safe storage condition for ginger preventing from beauvericin and enniatins contamination by *F. oxysporum*.

P1-28 Effect of Raw Material Management of Anchovy Sauce on Scombrotoxin Production during Fermentation

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***Introduction**:* Scombrotoxin (histamine) produced during fermentation and maturation of fish sauce poses potential risks associated with its consumption.

***Purpose**:* This study was conducted to establish a raw material management plan to reduce the level of scombrotoxin generated during the fermentation process of fishery sauce.

***Methods**:* To monitor the latest commercial products, fish sauce products from various countries (such as South Korea, Thailand, and Italy) were purchased and analyzed (n=45). In addtion, the freshness of raw materials was arbitrarily adjusted, and changes in scombrotoxin levels were tracked for about 120 days according to the fermentation progress. For the analysis of scombrotoxin, 1% chloroacetic acid solution was used for derivatization followed by HPLC for quantification.

***Results**:* The scombrotoxin concentration in most (approximately 80%) of commercially available fish sauce products was higher than the FDA guidelines of "500 mg/kg or less." The analysis of changes in scombrotoxin according to the freshness of raw materials showed that the concentration of raw materials (day 0) varied between 283.84±6.55 mg/kg for sample group A (good), 268.87±4.91 mg/kg for B (fair), and 470.56±1.16 mg/kg for group C (poor). In sample group A, scombrotoxin was 530.33±33 mg/kg on the 48th day and 1164.90±42.82 mg/kg on the 120th day. By contrast, scombrotoxin in group C exceeded 500 mg/kg in the early stage of fermentation (first 13 days), and 2111.42±54.18 mg/kg of excess scombrotoxin was produced on the 120th day. The lower the freshness of raw materials, more the amounts of scombrotoxin produced and faster the rate of increase in scombrotoxin levels.

***Significance**:* This study confirms that the freshness of raw materials of anchovies is related to the production of scombrotoxin, suggesting the importance of management of raw materials in the production of fermented anchovy foods. The results of this study can be applied to establish quality standards for raw materials in the future.

P1-29 Infiltration Potential of Pesticides in Banana during the Latex Removal Stage

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Introduction: Pesticide residues represent an important food safety concern and banana is one of the most highly consumed fruits worldwide.

Purpose: The effect of a latex removal step, in an aqueous environment, on the infiltration potential of the two pesticides with the highest probability and severity of occurrence as chemical hazards in banana was assessed.

Methods: Bananas (*Cavendish* variety), zero degree of maturity and 13 weeks of harvest, were provided by CORBANA (National Banana Corporation, Costa Rica). The two agrochemicals with the greatest severity and probability of presence were determined following the chemical risk assessment tool from World Health Organization. Also, the concentration of these compounds was determined in banana peels and pulp before and after water immersion using the QuEChERS method. Banana internal temperature, immersion water temperature and fruit immersion time were recorded. Three banana clusters, from independent banana branches, were tested per replicate. The experiment was performed in triplicate.

Results: From 119 compounds, bifenthrin and pyriproxyfen were identified as the pesticides with the highest probability and severity of occurrence in Costa Rican bananas. Both compounds were not detected in banana pulp after water immersion, suggesting that infiltration is not a concern under the studied conditions. The latex removal step did not significantly change the concentration of both compounds in banana peels, (*P = 0,6189*) for bifenthrin, and (*P = 0,8335*) for pyriproxyfen.

Significance: The findings are of relevance to banana industry. Despite pesticide infiltration may not represent a concern, pesticide concentration will not decrease after banana immersion.

P1-30 Method Development and Validation for the Determination of Ethylene Oxide and 2-Chloroethanol in Dried Raw Ingredients by GC-MS/MS

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Introduction: Analysis of ethylene oxide (EO), a major food safety risk when used as a fumigant to control pests, has challenges causing it to either interact with matrix components to form mainly, ethylene chlorohydrin (2-CE) or due to its high volatility, evaporates.

Purpose: Validate a method for the analysis of free EO and its metabolite, 2-CE in dried raw ingredients.

Methods: Sample extraction was performed using a QuEChERS method for analysis of EO and 2-CE on a GC-MS/MS in dried raw ingredients. Validation consisted of linearity, precision, accuracy, and limits of detection and quantitation using ginger root, sesame seeds and a proprietary blend. Sesame seeds and the blend were extracted nine times for precision. Ginger root was extracted and spiked four times with EO (10 and 100 ng/mL) and 2-CE (10, 100 and 1000 ng/mL) for accuracy. Ginger root (n=7) was used to determine LOQ for EO and 2-CE.

Results: Linearity of the method for EO and 2-CE was determined to be 5 to 100 ng/mL and 10 to 1000 ng/mL, respectively, both with $R^2 \geq 0.99$. Validation demonstrated precision of RSD < 20%. Recoveries for 2-CE were 103 to 130% but lower for the more volatile EO at 43.5 to 61.6%. LOQ for EO (8.23 ppb) was below the EU MRL of 10 ppb, and for 2-CE it was 16.7 ppb.

Significance: This study validated an optimized and robust analytical method for the quantification of EO and 2-CE in a single run. VDF FutureCeuticals will be able to monitor EO as the sum of EO and 2-CE to address the use of EO and increased public health concerns due to the risk of EO in food.

P1-31 An Evaluation of the Analysis for PFAS Using the FDA Protocol and Occurrence of PFAS in Food Contact Materials

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Introduction: PFAS, the forever chemicals, have been banned in certain situations from food contact materials (FCM), yet can find their way into foodstuffs in several other ways.

Purpose: To assess the various routes for the introduction of PFAS into the food chain and link the analytical methodology that can be used to assess the presence.

Methods: For foods the FDA protocol was used to determine concentrations of PFAS and the individual types of PFAS are detailed. Additionally, PFAS content of several different FCMs are determined along with a correlation to the PFAS seen in food versus those detected in FCMs. An additional set of tests were done to correlate the total organic fluorine content (as a measure of PFAS) in FCM to that determined in the corresponding food.

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Results: The frequency of occurrence of PFAS in food stuffs was, thankfully, low and therefore correlation to the detection found in associated FCMs was not strong. The detections observed in foods were low ranging from 0.2 ng/g to 0.5 ng/g. However while the results of the targeted compound work were not as compelling, the results of the TOF analysis indicted a higher presence of organic fluorine compounds than would have been predicted by the targeted compound analysis.

Significance: Targeted Compound PFAS analysis by the FDA protocol is the most appropriate for low level analysis of these potential contaminants but is limited in its application due to the targeted nature. Total organic fluorine proves to be a better measure for characterizing FCMs.

P1-32 Aconitine Poisonings from Imported Sand Ginger Powder in BC, Canada

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Introduction: Two individuals presented to hospital with symptoms of cardiovascular irregularity, dizziness, and vomiting. They had eaten home-prepared chicken that evening cooked with recently purchased powdered ginger which they reported tasted unusual. The cases were treated and released within 12 hours.

Purpose: Emergency physicians and Poison Control (PC) suspected aconitine poisoning. Here we describe successful coordination by PC, Public Health (PH), and Analytical Food Chemists investigating a toxic food-related outbreak.

Methods: PH collected a sample of sand ginger for testing and requested all remaining product be held pending results. Chemical analysis was performed with liquid chromatography.

Results: Toxic alkaloids aconitine at 0.1304 ± 0.0122 % (w/w) and hypaconitine at 0.0017 ± 0.0002 % (w/w) were found. PH/PC issued a public advisory five weeks after the illnesses. Further testing of additional product did not detect sand ginger. The retailer had prepared 70g packages labelled as sand ginger from a 1lb wholesale bag so in-store contamination could not be ruled out. The product was imported from China, no other imports were traced to the distributor. No other cases were reported in BC, but a similar outbreak occurred several months later in Ontario.

Significance: Sand ginger is prepared from the plant *Kaempferia galanga.* Commonly called monkshood or wolfsbane, *Aconitum spp.* can appear similar when dried and ground into powder, although smell and taste differ. Alkaloids in the roots and stems of *Aconitum* are highly toxic with doses of 1-2mg known to be lethal. The levels of alkaloids detected in the 'sand ginger' sample collected from the retailer in this case are at the higher end of the range reported in *Aconitum spp.* Testing was crucial in this event for PH to confirm the cause, issue an advisory and take further actions.

P1-33 Aflatoxin Contamination in Sesame

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Introduction: Sesame is a major oil-based seed crop that has been widely cultivated and consumed in Pakistan. Unfortunately, sesame is highly prone to aflatoxin contamination. Over half of the global population, mostly in developing countries, are at threat of chronic exposure to unknown levels of aflatoxin, which can be associated with reduced liver and kidney function, and immune system suppression. Sesame consumption and export is steadily increasing in Pakistan as well as globally mainly due to increasing health awareness.

Purpose: Pakistan is an exporting country of food to European countries, thus there is an interest to understand the occurrence of aflatoxin in Pakistani grains.

Methods: In the present study, 50 fresh and stored sesame seed samples were collected from the Sialkot, Pakistan. AF extraction and analysis were carried out using the Best Food method following standard protocol of the AOAC International and analyzed the aflatoxin level by HPLC. All the experiment was performed in triplicates. Two-way ANOVA followed by Duncan equation is used with significance of 0.05 ($P< 0.05$).

Results: The overall result reported that all the samples were contaminated with AFB_1, however AFB_2, AFG_1 and AFG_2 was not detected. AFB_1 concentrations were significantly higher ($P<0.05$) in stored samples than in fresh samples, with mean concentrations of 43.8 and 13.7 µgkg-1, respectively. Among these samples, 45% of fresh and 100% of stored samples contained AFB_1 more than 5.0 µgkg^{-1} and thus were not fit for human consumption as per maximum limit assigned by the European regulations (4µgkg^{-1})

Significance: This report is the first on the aflatoxin contamination in sesame seeds from Sialkot, Pakistan. These baseline data are an initial step in the effort to deal with this significant food safety issue. It is recommended be tested for aflatoxin in sesame seeds and other grains, to protect the consumers and to adhere to the European regulation.

P1-34 Results of a Multi-Year Inter-Laboratory Proficiency Testing Program for Aflatoxin in Corn

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Introduction: Proficiency testing programs are important to measure quality of results from HPLC and HPLC/MS determination of mycotoxins in ground corn.

Purpose: This research provides results from a double-blind study of accuracy, precision and reproducibility of aflatoxin quantitated in paired ground corn samples determined by HPLC and HPLC/MS over several years.

Methods: Double-blind ground corn samples with known amounts of aflatoxin were provided to each laboratory. Neogen's sample preparation process used 25 grams of sample extracted in 125 ml of 70% methanol/water. Filtered extracts were analyzed by HPLC-FLD in triplicate. Double-blind samples from the same lot of ground corn were analyzed once per year for four years. Statistical analysis including ANOVA was completed using Minitab 18 (Minitab, LLC).

Results: Six laboratories participated in the latest round and results from one laboratory were statistical outliers. Excluding those results, the inter-laboratory mean was 4.6±0.9 ppb aflatoxin (CV=18.7%, n=15) for a ground corn check sample containing nominal 4.8 ppb aflatoxin; 19.1±3.2 ppb (CV= 16.8%, n=15) for a nominal 19.2 ppb sample and 94.2±17.1 (CV=18.2%, n=15) for a nominal 87.9 ppb sample. Over four years of evaluation, nine laboratories participated. Excluding results from one laboratory there was no statistical difference in the mean for the 4.6 ppb sample and standard deviations (SD) were <1ppb for all laboratories except two. For the 19.1 ppb sample, SDs were <1.8 ppb except one lab which exceeded 3 ppb. For the 94.2 ppb sample, only one laboratory had results statistically different from the other lab means. Standard deviations were <11 ppb for all labs except two labs which exceeded 19 ppb.

Significance: The comparison of multi-year aflatoxin results provided information about the quality of the ground corn check samples and the long-term proficiency of the laboratories.

Poster

P1-35 Toxicity Studies of Phenolics and Phenolic-Branched Fatty Acids

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Introduction: Phenolic-branched chain fatty acids (phenolic BCFAs) have been synthesized as new bio-based antimicrobials; however, no published data on the safety of the phenolic BCFAs were available.

Purpose: This study was initiated to evaluate the developmental toxicity and estrogenic activity (EA) of four phenolic soy-BCFAs (with phenol, thymol, carvacrol, or creosote branches).

Methods: An *in silico* simulation Toxicity Estimation Software Tool (T.E.S.T) was conducted as the first step for toxicity prediction. Developmental toxicity of phenolic BCFAs was investigated via an *in vivo* chicken embryonic assay at an effective and relevant human exposure range (from 14.5 to 180 µg/kg, 8 eggs per group). Methyl-branched iso-oleic acid, phenol and creosote were included as controls. MCF cell proliferation assay was applied for EA detection at 0.1 to 10 µM.

Results: T.E.S.T results predicted that the phenolic BCFAs had much higher toxicities to aquatic organisms than free phenolics did, whereas the opposite was predicted for rats, where toxicity of phenolic BCFAs was lower than that of phenolics. Four phenolic soy-BCFAs showed variable degrees of developmental toxicity in the chicken embryonic assay (mortality rates ranged from 0% to 50%), with the creosote-soy BCFA which had the highest purity exhibiting the lowest mortality rate. Covalently bonding of creosote to fatty acids drastically reduced its developmental toxicity in the chicken embryo, and a non-monotonic dose response was observed in creosote and creosote-soy BCFA treatments. Comparable mortality rates were observed for thymol- and carvacrol-soy BCFAs, while carvacrol-soy BCFA significantly increased TBARs values ($P<0.05$). Additionally, the MCF-7 cell proliferation assay findings revealed that phenol-stearic BCFA showed EA while the phenol treatment had little EA at 10 µM.

Significance: Phenolic BCFAs have tremendous potential for commercial applications in food and healthcare industries. Performance of toxicological studies is necessary before these compounds can be considered for commercial applications.

P1-36 Effect of Amino Acids Addition in Thermal Processing of Foods on Alleviating Acrolein-Induced Inflammation in Kupffer Cells

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Introduction: Acrolein exists widely in heat-processed foods such as bakery products and is a toxic substance that easily reacts with proteins, DNA, nucleic acids and other substances in the human body.

Purpose: This study investigated the effect of thermal reactants (also called adducts) from acrolein or acrolein-amino acid on the inflammation in hepatic Kupffer cells.

Methods: In this study, the acrolein (50 to 200 µM), selected amino acids (glycine, alanine, valine, cysteine, glutamine, threonine and glutamic acid, 500 µM), and acrolein (50 µM)-amino acid (500 µM) solutions were refluxed at 170°C oil bath, and the supernatants were then collected and freeze dried to obtain thermal reactants. The cytotoxicity (MTT) test of these thermal reactants on Kupffer cells was performed. The expression of inflammation-related proteins of Kupffer cells were analyzed by Western blot.

Results: The results suggested that the 50 µM acrolein thermal reactant suppressed approximately 20% viability of Kupffer cells. The results also found that the heating of acrolein-cysteine significantly reduced the residual amount of acrolein after refluxing, and showed the highest cell survival rate of Kupffer cells compared to the other acrolein-amino acid groups. Western blot analysis found that acrolein-cysteine thermal reactant decreased the expression of inflammation related proteins such as IKKβ, Nf-κB, caspase-1, pro-caspase-1 and NLRP3 in Kupffer cells compared to the acrolein thermal reactants.

Significance: The above observations demonstrated that some amino acids such as cysteine may react with acrolein to form adducts easily during heat processing and alleviates the cytotoxicity and inflammation of acrolein on Kupffer cells. The addition of amino acids in food production may reduce the damage of acrolein from heat processing in the human body.

P1-37 Food Toxicological Evaluation of Edible Insect *Locusta migratoria* as an Alternative Food Resource with Antibacterial Properties and Functional Nutrients

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Introduction: Edible insects are increasingly recommended as alternative food resources to meet the nutritional needs. Previously, we observed that the addition of edible insect powders to bread with high water-activity suppressed the growth of general viable bacteria, particularly *Staphylococcus aureus*, but their food toxicological properties have not been fully evaluated.

Purpose: The toxicological properties of the powders of migratory locust (*Locusta migratoria*, ML), a representative edible insect, were biochemically and histopathologically evaluated in rats.

Methods: In an acute toxicological study, Wistar male rats aged 5 weeks-old (n = 7/group) were orally administered with 10 and 20 g/kg b.w., and then their general condition and body weight were monitored for 2 weeks. In a sub-chronic toxicological study, Wistar male and female rats aged 5 weeks-old (n = 12/group) were divided into three groups (control, 1%-, and 3%-ML groups) and fed with control, 1%- and 3%-ML containing diets, respectively, for 28 and 90 days. Hypertrophy of organs were verified, and biochemical, hematological, and histopathological parameters in blood, liver, and digestive tracts were evaluated. Statistical analyses were determined by Dunnett tests ($p<0.05$).

Results: The acute toxicological study observed steady weight gain of rats without death and other adverse effects by the dietary ML, and LD_{50} value was more than 20 g/kg b.w. The sub-chronic toxicological study also did not show that dietary ML induced organ hypertrophy and adverse effects in blood, plasma, liver by biochemical, hematological, and histopathological analyses, although the cecal short-chain fatty acids was suppressed by dietary ML because of their antibacterial properties.

Significance: Since ML powders have been recognized as alternative food protein materials with n-3 lipids (43% of total fatty acids) and chitin fibers (10% in total powder), the present toxicological findings can contribute the spread of edible insect foods with safety and antibacterial functionality.

P1-38 Growth of *Listeria monocytogenes* in the Presence of Enoki Mushrooms

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Introduction: *Listeria monocytogenes* has been recently linked to outbreaks from enoki mushrooms. Surveillance efforts, initiated after the 2020 outbreak, identified that 43% of the enoki mushroom entries imported from Korea and sampled at the port-of-entry by FDA were contaminated with *L. monocytogenes*. These observations led to questions regarding when and how *L. monocytogenes* was contaminating enoki mushrooms.

Purpose: This study evaluated the potential for *L. monocytogenes* to co-exist in culture with enoki.

Methods: Individual and co-culture trials were performed in potato dextrose broth (PDB) at 22°C. *L. monocytogenes* counts were determined via colony counts while enoki growth was subjectively observed for changes in the mycelium bulk. These cultures were used to inoculate a soy-hardwood substrate.

Substrate cultures were incubated at 22°C until mycelial infiltration was complete. The substrate cultures were then scraped and transferred to 16°C at 83% humidity for fruiting body generation, or pinning, assessed via visual examination. Growth was also assessed via turbidity in a Bioscreen C at 22°C.

Results: *L. monocytogenes* could not grow in PDB as an individual culture but grew in the presence of enoki, from ~1.7×10^5 CFU/mL to a peak >10^7 CFU/mL between days four and eight. During this time, mycelia bulk increased within the PDB culture. After the peak, the *Lm* population declined one log by day 14, while mycelia continued to expand. Two independent trials were performed, inoculating the substrate with 14-day cultures, and found no delay in mycelial infiltration, compared to parallel, uncontaminated enoki cultures.

Significance: These results indicate that enoki can grow with *L. monocytogenes* and potentially produce mature mushrooms. Further study is needed to look at the impact of differing levels, times, and regions of contamination on enoki.

P1-39 Modeling the Fate of *Listeria monocytogenes* and *Salmonella enterica* on Fresh Whole and Chopped Wood Ear and Enoki Mushrooms

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Introduction: Recent listeriosis and salmonellosis outbreaks associated with imported specialty mushrooms in the U.S. have prompted research to understand how foodborne pathogens survive in these unique matrices.

Purpose: To evaluate the growth kinetics of *Listeria monocytogenes* and *Salmonella enterica* on whole and chopped wood ear and enoki mushrooms.

Methods: Fresh mushrooms were either chopped or kept whole and inoculated with a four-strain cocktail of either *L. monocytogenes* or *S. enterica* at 3 log CFU/g. Inoculated mushrooms were stored at 5, 10 or 25°C for up to 7 d. Populations were enumerated at 0, 1, 3, 5 and 7 d. Three independent trials with triplicate samples for each timepoint were conducted. Population data for each pathogen were fitted to the Baranyi model via DMFit to estimate growth rates. Population differences were statistically compared using ANOVA, whereas growth rates were compared using ANCOVA; $p<0.05$ was considered significant.

Results: No proliferation was observed for either pathogen on whole or chopped wood ear or enoki mushrooms at 5°C. At 10°C, moderate growth was observed for both pathogens on enoki mushrooms (0.12-0.28 log CFU/g/d) and for *L. monocytogenes* on wood ear mushrooms (0.03-0.09 log CFU/g/d). Both pathogens proliferated at 25°C on both mushroom types and preparations. The growth rates ranged from 0.43 to 3.27 log CFU/g/d, resulting in 1 log CFU/g increases in only 0.31 d (7.44 h) to 2.32 d, with more rapid growth rates observed on enoki mushrooms.

Significance: The results of this study provide information on the growth of foodborne pathogens in specialty mushrooms and can inform discussions surrounding the safe time and temperature conditions for these food commodities.

P1-40 Population Dynamics of *Salmonella enterica* and *Listeria monocytogenes* during Rehydration of Dehydrated Enoki Mushrooms and Subsequent Storage

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❖ ***Developing Scientist Entrant***

Introduction: Recent listeriosis and salmonellosis outbreaks in the U.S. have been associated with imported specialty mushrooms. These mushrooms are commonly sold fresh or dehydrated. This study evaluated the survival and growth of two foodborne pathogens on dehydrated mushrooms during both rehydration and storage.

Purpose: To analyze the population dynamics of *S. enterica* and *L. monocytogenes* during rehydration of dehydrated mushrooms and subsequent storage at various temperatures.

Methods: Fresh enoki and wood ear mushrooms were dehydrated for 24 h at 64°C. Dehydrated mushrooms were inoculated with a four-strain cocktail of *S. enterica* or *L. monocytogenes* at 4 log CFU/g. Mushrooms were dried for 1 h, followed by rehydration for 2 h with 25°C water. Rehydrated mushrooms were stored at 5, 10, or 25°C for up to 14 d. The pathogens were enumerated at 0, 1, 3, 6, 9 and 14 d. Three independent trials with triplicate samples at each timepoint were completed. Population differences were evaluated via Student's t-test; $P<0.05$ was considered significant.

Results: S. enterica and *L. monocytogenes* populations after rehydration were 2.78±0.11 and 3.17±0.25 log CFU/g, respectively. There was no significant change in population for either pathogen before or after rehydration. While *S. enterica* survived but did not proliferate on enoki mushrooms stored at 5°C, the population increased significantly by 4.23 and 5.36 log CFU/g at 10 and 25°C after 14 d, respectively. *L. monocytogenes* proliferated at all temperatures, with significant population increases of 0.51, 2.65, and 3.50 log CFU/g at 5, 10, and 25°C, respectively.

Significance: This study outlines the importance of refrigerated storage temperature for rehydrated enoki mushrooms and can inform discussions on the safe time and temperature combinations for safety.

P1-41 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 Antibiotic resistance (ABR) bacteria 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 isolate gram-positive and gram-negative bacteria from street vended foods and fresh vegetables and examine the antibiotic susceptibility of the isolates.

Methods: Biochemical and morphological identification of the 35 collected isolates from fuchka, chotpoti, and salad was done by the API (Analytical Profile Index) 20E test and colony characteristics. Then their susceptibility to 11 different antibiotics was investigated by the disk diffusion method.

Results: Among *E. coli*, *Klebsiella*, and *Staphylococcus aureus*, almost all of them exhibited multidrug resistance (MDR). All isolates were 100% sensitive to Aztreonam and Ceftriaxone. About 100% of *E. coli* and *Klebsiella* strains were resistant to Cefuroxime and Oxacillin. Furthermore, all *Staphylococcus aureus* strains were resistant to Oxacillin.

Significance: The study raises concern about the safety of street vended foods, and warns about the lesser-known transmission of resistant bacteria to humans.

Poster

P1-42 Fate of *Listeria monocytogenes* in Ready-to-Eat Leafy Green Salads during Refrigerated and Frozen Storage

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✦ ***Undergraduate Student Award Entrant***

Introduction: In 2021, a multistate listeriosis outbreak was linked to ready-to-eat packaged leafy green salads. Fate of *Listeria monocytogenes* (*Lm*) in leafy green salads could help us better interpret enumeration results and understand the risk associated with *Lm* contamination in leafy green salads.

Purpose: This study investigated how the storage of ready-to-eat packaged leafy green salads at 4°C and -30°C affected the survival and growth of *Lm* for up to 60 days.

Methods: A *Lm* strain previously isolated from naturally contaminated lettuce was artificially inoculated to fresh leafy green salads at a level of 1.87 log CFU/g, before storage at 4°C and -30°C for up to 60 days. On the sampling day, 3 replicates of 25 g were blended with 225mL of buffered *Listeria* enrichment broth and directly enumerated on *Listeria* selective agar plates. Most probable number (MPN) was also performed following the FDA *Bacteriological Analytical Manual* protocol. Uninoculated salads were stored at 4°C and sampled on the sampling day to quantify the aerobic bacteria using 3M™ Petrifilm™ Aerobic Count Plate.

Results: During the storage at 4°C, increasing levels of spoilage and decomposition were observed throughout the 60 days. The level of aerobic bacteria in uninoculated salads increased from 7.23 to 8.70 log CFU/g during that time. In inoculated samples, *Lm* level decreased slightly from Day 0 to 14, before increasing steadily to finally reach 4.94 log CFU/g on Day 60. Thus, *Lm* was able to proliferate at 4°C regardless of the presence of competitive microflora. Conversely, *Lm* level remained unchanged during storage at -30°C for 60 days

Significance: Lm can survive in packaged leafy green salads during frozen storage for at least 60 days and can proliferate during refrigeration, regardless of the presence of aerobic microflora. These results help interpret *Lm* enumeration results in naturally contaminated samples.

P1-43 Genomic Characterization of Competitive Exclusion *Lactobacillus salivarius* Strains Isolated from Poultry

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Introduction: Competitive exclusion (CE) has been an important strategy in prevention and control of the colonization of poultry by foodborne pathogens *Campylobacter jejuni* and *Salmonella enterica*. Three strains of *Lactobacillus salivarius* have shown potential as CE for poultry based on in vitro assays and poultry challenge trials. A genomic characterization of these strains may provide further insights on the working mechanism of potential CE strains.

Purpose: This study aimed to characterize three CE *Lactobacillus salivarius* strains through whole genome sequencing (WGS) and analysis.

Methods: Genomic DNA was extracted from pure culture using DNeasy blood and tissue kit. Whole genome shot gun sequencing was performed by Illumina NextSeq 500 with DNA libraries prepared using the Nextera XTDNA kit. Raw reads were trimmed, and quality checked by Trimmonatic and FastQC, respectively. De novo assembly was performed using SPAdes. Genome annotation was done using Prokka. Additionally, antibiotic resistant profiles were predicted using Resistance Gene Identifier (RGI). The detection of secondary metabolite biosynthetic gene clusters and bacteriocins was performed using antiSMASH and Bagle 4, respectively.

Results: No antibiotic resistant gene was detected in one of the three strains whereas only resistance genes for tetracycline detected in another strain and resistance genes for erythromycin and tetracycline in the third stain. In addition, genes encoding several bacteriocins including enterolysin A and salivaricin P were detected in these strains.

Significance: This study demonstrated that WGS level characterization of CE bacteria can provide significant information about their effectiveness and safety for their application in the industry. The strain lacking antibiotic resistance gene could be a better choice in such application.

P1-44 Combination Treatment of Bacteriophage and Essential Oils to Inactivate *Salmonella* Enteritidis on Quail Egg

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Introduction: *Salmonella* Enteritidis (*S*. Enteritidis), which is one of the main foodborne pathogens causing food poisoning in human by consuming poultry product or migrating from food contact materials, can cause serious problems to food safety and customer health.

Purpose: This study was aimed at evaluating the efficacy of *S*. Enteritidis reduction by using bacteriophage (BP) and essential oils (EOs) on quail eggs.

Methods: Two EOs (thymol [Thy] and geraniol [Ger]) and BP (CAU-SEP-3) were treatments in this experiment. After attachment of bacteria on quail egg, minimal inhibitory concentration (MIC) level of EOs and multiplicity-of-infection (MOI) 100 of CAU-SEP-3 were treated at 25 °C and 4 °C for 7 days. Stored samples were homogenized with stomacher for 2 min, subsequently, samples were diluted and spread on media. To visualize and quantify control and combination-treated *S*. Enteritidis, confocal laser scanning microscope (CLSM) and COMSTAT software were utilized.

Results: On quail egg at 25 °C , *S*. Enteritidis was reduced by 2.03, 2.55, 2.45, 2.92 log CFU/egg in treatment of BP, BP+Thy, BP+Ger, and BP+Thy+Ger, respectively. At 4°C , *S*. Enteritidis was reduced by 1.01, 1.06, 1.13, 1.33 log CFU/egg in BP, BP+Thy, BP+Ger, and BP+Thy+Ger treatment. The side and top view of CLSM image of *S*. Enteritidis biofilm also showed that combination treatment at 4 °C had the highest efficacy for inactivating *S*. Enteritidis biofilm. COMSTAT analysis represented similar result with CLSM image that combination treatment at 4 °C showed the most reduction of *S*. Enteritidis biofilm on quail egg.

Significance: These results demonstrated that combination treatment of BP and EOs was effective method for inactivating *S*. Enteritidis on quail's egg. Moreover, this method can be utilized to prevent cross contamination of *S*. Enteritidis in an egg processing factory.

P1-45 Potential Enhanced Heat Tolerance of *Salmonella* I 4,5,[12]:I:- from a Roast Pork Outbreak in 2015

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* isolates from foodborne outbreaks can have enhanced tolerance to processing stresses, including heat, as seen in the 2013-2014 *Salmonella* Heidelberg outbreak in poultry.

Purpose: To determine whether multidrug resistant isolates of *Salmonella* I 4,5,[12]:i:- from a roast pork outbreak in Washington State in 2015 had enhanced tolerance to heat stress (56°C).

Methods: Six *Salmonella* I 4,5,[12]:i:- isolates from the 2015 roast pork outbreak were heat-shocked at 56°C. Aliquots were taken at the start of stationary phase and serially diluted into phosphate-buffered saline. Aliquots were then incubated at 56°C and pour-plated at 0, 3, 6, 9, 15, 30, 45, and 60 minutes post heat-shock. Plates were incubated at 37°C for 36 hours, after which colony counts were recorded. Statistically significant differences between isolates were determined via analysis of variance (ANOVA) with Tukey's HSD test, with significance defined at $p_{adj} < 0.05$.

Poster

Results: After the initial period of 3 minutes, all isolates showed a significant decrease in growth, and at 30 minutes post-scald all isolates plated were below detectable limits (less than 1 CFU/mL) ($p_{adj} < 0.05$). Isolate 14 had significantly lower heat tolerance than isolates 11-16. The other isolates displayed similar heat tolerance to each other, and did not vary much in their tolerance. Isolate heat tolerance was not associated with sanitizer tolerance; isolate 14 was previously found to have high MICs for quaternary ammonium compounds and sodium hypochlorite, but the lowest heat tolerance.

Significance: Understanding processing stress tolerance in *S. enterica* is critical to understanding how to mitigate *S. enterica* survival in food industry and to developing standard operating procedures to prevent future outbreaks.

P1-46 Fate of *Listeria monocytogenes* during Storage of Hard-Boiled Eggs Following Treatment with Organic Acids

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Introduction: RTE, peeled, hard-boiled eggs (HBEs) are a common product for retail and commercial use. Citric acid is commonly used to control *L. monocytogenes* during HBE storage, however recent outbreaks have prompted research examining the effectiveness of this antimicrobial.

Purpose: To assess the ability of citric and other organic acid treatments pre- and post-inoculation to eliminate low levels of *L. monocytogenes* on HBEs through 28 d storage at 5°C.

Methods: For pre-treatment inoculation trials, HBEs were dip-inoculated with *L. monocytogenes* for 20 min, dried for 10 min, followed by treatment with 2% citric, acetic, lactic, or malic acid for 24 h at 5°C. For post-treatment trials, HBEs were soaked in 2% acid for 24 h at 5°C, dried for 10 min, spot inoculated, and dried for 20 min. All HBEs were stored individually in bags at 5°C for up to 28 d. Triplicate HBEs were assessed at each timepoint and three independent trials were conducted. The presence of *L. monocytogenes* was determined via enrichment and expressed as n/9. Fisher's exact test was used to determine statistical significance; $P<0.05$ was considered significant.

Results: The initial population of *L. monocytogenes* on the HBEs was 1 log CFU/egg. For pre-treatment trials, *L. monocytogenes* was not detected on HBEs after 1, 7, and 28 d with acetic, malic, and citric acids, respectively. Lactic acid was not effective as detection occurred through 28 d (3/9) with no significant decrease. For post-treatment contamination, *L. monocytogenes* was not detected on HBEs after 7 d when treated with citric acid. The pathogen survived through 28 d when HBEs were treated with acetic (2/9), lactic (4/9), and malic (3/9) acids, with no significant decrease in detection.

Significance: The results of this study indicate that organic acids may be effective at reducing low levels of *L. monocytogenes* on HBEs during storage.

P1-47 Assessment of Population Stability of *Salmonella enterica* in Matrices for Use in Dry Inoculations

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Introduction: Challenge tests associated with fresh produce generally utilize liquid bacterial inoculation methods. However, a recent salmonellosis outbreak in the U.S. was linked to peaches which were thought to be contaminated via dust from a nearby farm. This study examined the population stability of *Salmonella enterica* in different dry matrices to evaluate their use for dry transfer dust inoculations of produce.

Purpose: To examine the survival of *S. enterica* during storage of three different dry matrices for use in dry dust inoculations of produce.

Methods: A four-strain cocktail of *S. enterica* (10 mL) was inoculated into 50 g of silica (70-230 mesh, Aw=0.224), sand (50-70 mesh, Aw=0.228), or corncob small animal bedding (ground to 30 mesh, Aw=0.050). Matrices were mixed by hand and stored at 22°C/30%RH for 90 d. Every 7 d, the Aw of the matrices was measured and *S. enterica* was enumerated. Three independent trials were conducted with triplicate samples. Differences in Aw of the matrices or *S. enterica* population were statistically compared using Student's t-test; $p<0.05$ was considered significant.

Results: The population of *S. enterica* (10.88±0.54 log CFU/g) and Aw (0.740±0.150) of the three matrices were not significantly different post-inoculation. After 7 d storage, the Aw of the matrices equilibrated to their pre-inoculation values. After 28 d, the *S. enterica* population in the bedding (10.74±0.07 log CFU/g) was significantly higher than in the silica and sand (9.44±0.38 and 9.70±0.01 log CFU/g, respectively). After 90 d, the population of the pathogen in bedding and sand (9.82±0.49 and 9.37±0.18 log CFU/g) were both significantly higher than in silica (8.59±0.46 log CFU/g). The *S. enterica* population was most stable in bedding during storage, only reducing by 1.06 log CFU/g after 90 d.

Significance: The results of this study can aid in determining the most appropriate dry matrices to mimic dry dust inoculation of produce.

P1-48 Evaluation of the Phagedx™ *Salmonella* Assay for the Detection of *Salmonella* in Raw Ground Turkey

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Introduction: The PhageDx™ *Salmonella* Assay is a reporter-phage based rapid detection method that detects viable *Salmonella* spp. in food in as little as 9 hours.

Purpose: The performance of this alternative method was compared to the Canadian culture reference method MFHPB-20, the VIDAS® UP *Salmonella* Phage Technology (SPT) assay (BioMerieux), and the BAX System *Salmonella* Real-time (RT) PCR Assay (Hygiena).

Methods: Raw ground turkey derived from a *Salmonella*-free flock was inoculated with a 3-strain *Salmonella* cocktail at three levels: 20 samples at low level (0.2-2 CFU/test portion) intended to give fractional positive results, 5 samples at high level (2-5 CFU/test portion), and 5 un-inoculated control samples. After a 48h cold stress acclimatization period, 375g portions were homogenized with 1,125mL of BPW, incubated at 41 ˚C and tested by the three assays at 7h and 18h of incubation. All enrichments were confirmed on culture media by the MFHPB-20 reference method at 24h of incubation.

Results: Statistical analysis of fractional positive paired results was performed according to McNemar's Chi-square formula (ISO 16140). A statistically significant difference was observed between the BAX RT PCR and the VIDAS SPT when tested at 7 hours of incubation compared to the reference method MFHPB-20. Neither the PhageDx™ assay tested at 7h or 18h of incubation nor the VIDAS SPT or BAX RT PCR Assays conducted at 18h of incubation showed statistically significant differences to the MFHPB-20 reference method.

Significance: The results of the three assays were statistically equivalent at the 18h test point and when compared to the culture reference method MFHPB-20. In addition, the PhageDx™ *Salmonella* assay was able to detect the target at 7h. This highlights its potential as a valuable tool for detecting *Salmonella* in raw ground turkey in real-world settings, as it can save a significant amount of time during detection.

P1-49 *Staphylococcus aureus* Survival and Growth in Doughs and Batters

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Introduction: Ingredients used in the production of doughs and batters are known to be microbiologically sensitive. *Staphylococcus aureus* is a pathogen with a well-documented history of causing outbreaks in bakery products and is of particular concern due to its ability to produce heat stable enterotoxins. It is important to understand the hold time for high water activity doughs and batters to ensure toxins will not exist in the final product.

Purpose: S. aureus inoculated doughs and batters were held at various temperatures for up to 8 days to determine if *S. aureus* growth would be achieved at a level that would produce toxins.

Poster

Methods: Two doughs and two batters were inoculated with low levels of a *S. aureus* cocktail (NCC 8205 (SEE), NCC 8202 (SED), and NCC 8231). The doughs and batters were incubated between 25 and 35°C and plated on Baird-Parker agar for enumeration on days 0, 2, 4, 6, 7, and 8. The study was completed in triplicate.

Results: The pH of the doughs and batters varied between 6.63 and 7.16. The Aw varied between 0.85 and 0.88 for all 4 doughs and batters over the course of the 8-day study. The starting concentrations of *S. aureus* were below 4 log CFU/g for all doughs and batters. *S. aureus* increased the most in Dough A with the average starting concentration of 3.86±0.07 log CFU/g and ending at 8.14±0.18 log CFU/g after being held at 26°C for 8 days. *S. aureus* was still detected in all doughs and batters after 8 days.

Significance: Holding times are critical to some of the doughs and batters tested as *S. aureus* grew to levels that would lead to the production of toxins which would be present in the finished product.

P1-50 Isolation and Characterization of *Salmonella* and *E. coli*-Specific Bacteriophages Collected from Minnesota Waste Water Treatment Plant

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* and *E. coli* are one of the main causes of foodborne illnesses in the United States, despite having numerous bacteria control methods in the industry, foodborne outbreaks continue to be a persistent public health concern. Bacteriophages have shown promising results against bacterial growth and represent a novel approach as a food biocontrol agent.

Purpose: The objective of this research was to isolate, analyze, and describe the phenotypic attributes and genomic data of wastewater-isolated *Salmonella* and *E. coli* bacteriophages.

Methods: All six phages were isolated from a local Minnesota Wastewater Treatment Plant, using *Salmonella* Typhimurium as a host strain. Forty-seven bacterial strains, among them *Salmonella* and Shiga-toxin-producing *E. coli* strains, were used to determine the host range of the phages through a lawn plaque assay. The genomes of our bacteriophages were sequenced and annotated using bioinformatics tools. Transposon mutagenesis of *Salmonella* was used to identify mutations that confer resistance to the phages. Genetic ancestry and homology among these bacteriophages were obtained upon analysis of conserved DNA polymerase sequences.

Results: Six lytic *Epseptimavirus* bacteriophages specific to *Salmonella* and *E. coli,* including pathogenic *E. coli* O157:H7 strains, were isolated and identified. Bacteriophages EH2 and EH5 share the highest percentage of homology. Most of the *Salmonella* strains tested (51.85%) showed sensitivity towards our bacteriophages. A gene encoding a B12 vitamin-importing porin (*BtuB*) was determined to be a putative receptor for all six phages through transposon mutagenesis. The outcome of this research highlights the use of bacteriophages as a food processing aid to control pathogens.

Significance: This work demonstrates the potential application of bacteriophages for bacterial growth inhibition which can be a useful tool in the food industry to reduce the presence of foodborne pathogens.

P1-51 Survival of *Listeria monocytogenes* on Stainless-Steel Coupons within Dust Particles

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Listeria monocytogenes* can be deposited by dust and survive on surface areas that are difficult to sanitize. The role of humidity in the re-introduction of bacteria through dust and condensate/humidity needs to be explored to control contamination in packing houses.

Purpose: The objective of this study was to determine the survival of *L. monocytogenes* 4b and *L. innocua* (as surrogate) within dust on stainless-steel surfaces at two temperature and humidity conditions over 10 days.

Methods: Autoclaved dust (1 gram) containing ~7 log CFU/mL of overnight grown *L. monocytogenes* or *L. innocua* were inoculated on 3×3 cm^2 stainless-steel coupon surfaces held within sterile petri-plates at room temperature (RT; 52% relative humidity (RH)) or at refrigeration (4°C; 83% RH) over 10 days. Bacteria were recovered after serially diluting ten-fold in phosphate buffered saline and surface spread plating on Tryptic Soy Agar plates. Colonies were enumerated after incubation for 24 to 48 h at 37°C. Data from triplicate trials were statistically analyzed using JMP.

Results: Recovered *L. monocytogenes* at RT (22.5°C; 52% RH) were 7.06±0.38 log CFU/coupon at day 0, with significantly lower recovery of 3.97±0.72 log CFU/coupon after 10 days (~3.09 log reduction; p≤0.05), while *L. innocua* recovered were 6.37±0.45 log CFU/coupon at day 0 and 3.42±0.23 log CFU/coupon after 10 days (~2.95 log reduction; P≤0.05). Recovered *L. monocytogenes* at 4°C and 83% RH were 7.13±0.29 log CFU/coupon at day 0 and 7.0±0.58 log CFU/coupon after 10 days (insignificant 0.13 log reduction; p>0.05), while *L. innocua* recovered was 6.69±0.79 log CFU/coupon at day 0 and 5.87±0.21 log CFU/coupon after 10 days (insignificant 0.82 log reduction; p>0.05). Overall, both bacteria showed higher ability to survive at low temperature and high humidity.

Significance: Low temperature, high humidity and dust particulates that trap microorganisms play key roles in survival of *L. monocytogenes* and ability to transfer to foods.

P1-52 Isolation and Characterization of Bacteriophage Cau_VPP01 Specific for *Vibrio parahaemolyticus* and Their Application on *Vibrio* Cocktail Biofilm to Inhibit Seafood Contamination

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Introduction: *Vibrio parahaemolyticus* is a major foodborne pathogen that occurs mainly in shellfish and seafood. Bacteriophage is a naturally occurring bacterial virus that may infect specific host bacteria and is thought to be a potential antimicrobial food intervention due to their capacity to render target bacteria inactive in various settings.

Purpose: The purpose of this study was to isolate *V. parahaemolyticus* lytic bacteriophages in natural environments, analyze their characteristics, viability, and genome sequence and investigate the reduction effect of *V. parahaemolyticus* biofilms as a biological treatment agent in seafood.

Methods: Bacteriophage specific for *V. parahaemolyticus* was isolated from a natural environment. Characteristic analysis was conducted by host range determination, adsorption, and one-step growth kinetic. Viability analysis was performed by pH and heat stability. Genome sequencing was conducted by proteomic analysis, phylogenetic analysis, and DNA mapping. After that, the surface of squid and mackerel (2 x 2 cm, 5 g) was spot inoculated with 100 µL of *V. parahaemolyticus* and then 300 µl of bacteriophage lysate was dispensed.

Results: CAU-VPP01 bacteriophage showed that more than 80% of the phages were readily adsorbed to host bacteria in 10 min. Based on step growth kinetic result, the latent period was 20 min and the burst size was 20 PFU/cell. The specificity against *V. parahaemolyticus* were able to survive in wide range of pH and heat. The isolated bacteriophage was determined to belong to the siphoviridae family, and infect hosts from the Gammaproteobacteria group, which includes the vibrionaceae family. The bacteriophage was able to inhibit the value of biofilm for six hours and showed 1.4, 1.12 log CFU/cm^2 at 4 °C , 2.19, 1.74 log CFU/cm^2 at 15 °C on squid and mackerel, respectively.

Significance: This study was indicative that environment-derived *V. parahaemolyticus*-specific bacteriophage are effective in controlling *V. parahaemolyticus* biofilms in various seafoods at various conditions (refrigerated to room temperature).

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P1-53 Effects of Combined Treatments of Baicalin and Carvacrol on Reduction of *Salmonella* Typhimurium Biofilm Formed on Food Contact Surfaces

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Introduction: *Salmonella Typhimurium* (*S*. Typhimerium) is a Gram-negative, facultative anaerobic pathogen. *S*. Typhimerium has been considered as an important bacterial pathogen due to the formation of their biofilms which are potential sources for pathogens to contaminate fresh product.

Purpose: The aim of this study was to investigate anti-bioflim activity of baicalin and carvacrol and synergistic effects of two chemicals on biofilm formed by *S*. Typhimerium on three different food contact surfaces (stainless steel[s.s], polyethylene terephthalate[pet], rubber).

Methods: Minimum inhibitory concentration (MIC) of baicalin and carvarol was performed. Then, four different concentrations (0.5, 1, 2, 4 MIC) was exposed to *S.* Typhimerium biofilm with single and combined methods. Field emission scanning electron microscopy (FE-SEM) and confocal laser scanning microscopy (CLSM) were used to visually confirm the reduction effect.

Results: Baicalin and carvacrol both showed antimicrobial activity on food contact surfaces (s.s, pet, rubber). Baicalin reduced 0.67 CFU/cm^2, 1.2 CFU/cm^2 and 0.49 CFU/cm^2 biofilm formed on three surfaces (s.s, pet, rubber) respectively. Carvacrol reduced 1.15 CFU/cm^2, 1.58 CFU/cm^2 and 0.3 CFU/cm^2 biofilms formed on the s.s, pet, and rubber respectively. In the combination test, baicalin (0.5 MIC) with carvacrol (2 MIC) showed the effective reduction for inhibiting *S*. Typhimerium biofilms, showing 4.11 CFU/cm^2 to 4.92 CFU/cm^2 reduction, respectively.

Significance: This study demonstrated that baicalin had ability for preventing *S*.Typhimurium forming biofilm and more effective when used with carvacrol.

P1-54 Samplezyme: A Technological Breakthrough for the Biofilm Sampling on Food Contact Surfaces

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Introduction: Surface sampling of microorganisms encapsulated in biofilms is barely or not effective with current sampling methods.

Purpose: An enzymatic biofilm hydrolyzing solution (SampleZyme) was developed 1) to make the bacteria accessible to surface sampling and 2) to ensure compatibility with analytical methods used in food microbiology.

Methods: The efficacy of the enzymatic solution to hydrolyze biofilms was carried out with the microplate model described by Iglesias et al. (2019) and adapted to include bacterial strains encountered in agri-food industry (*Lactococcus lactis* and *Pseudomonas fluorescens*). Conventional cleaning solutions (sodium hydroxide (1%), phosphoric acid (1%) and chlorinated alkaline (1%)) were compared to SampleZyme. Inside the same plate, all solutions were tested six times and each plate was tested three times. The compatibility on analytical methods commonly used in food microbiology (bioMérieux VIDAS, Bio-Rad IQ Check , bioMérieux Tempo, selective media, Total flora ISO 4833) was tested with and without the SampleZyme solution on three strains of *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli* O157 and *Pseudomonas aeruginosa*. Each test was repeated five times. A total of 480 tests were performed. All the results obtained were analyzed statistically with nonparametric tests (Kruskal-Wallis and Mann-Withney tests).

Results: SampleZyme improves the removal of tested biofilms with a significant difference compared to sodium hydroxide (P<0.05; n=64), phosphoric acid (P<0.05; n=58) chlorinated alkaline detergent (P<0.05; n=66). SampleZyme and sodium hydroxide were the most effective at removing biofilms, other solutions are less or not at all effective. No statistical difference is observed in the results of the analytical methods with and without SampleZyme on *Salmonella* spp. (P>0.05; n=120), *Listeria monocytogenes* (P>0.05, n=150), *Escherichia coli* O157 (P>0.05, n=120) and *Pseudomonas aeruginosa* (P>0.05, n=90).

Significance: SampleZyme is a new tool to improve biofilm sampling on surfaces to assess the microbial quality and safety in the environment of agri-food industries.

P1-55 Synergistic Action of UV-C Assisted Postbiotic(J.27) to Eradicate *Salmonella* Biofilms on Food Contact Surfaces

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Introduction: Foodborne pathogens cause millions of cases of human illness and economic loss worldwide and contamination of poultry in the food industry, and poultry- related products by pathogenic bacteria such as *Salmonella* spp. is a common problem in developed and developing countries.

Purpose: The purpose of this study was to investigate the synergistic action of UV-C assisted postbiotic (J.27) against *Salmonella* Typhimurium and *Salmonella* Thompson biofilm formed on food contact surface (stainless steel [SS], silicone rubber [SR]).

Methods: *S.* Typhimurium and *S*. Thompson biofilm were formed on SS and SR for 24 h at 37 °C (Both food contact surfaces were prepared to 2×2×0.1 cm). After biofilm formation, the biofilms were treated with combination of UV-C (1 to 5 min) and postbiotic J.27 (1 to 4 MIC). The biofilm cells on treated food contact surfaces were detached by vortex in conical tube with 0.1% PW and glass beads. The detached biofilm cells were enumerated using plate count method.

Results: The biofilm on food contact surface was not greatly reduced by single treatment of J.27 or UV-C. Single treatment of J.27 (1-4MIC) reduced 0.79 to 1.52 log CFU/cm^2 of *S*. Typhimurium biofilm and 0.23 to 1.23 log CFU/cm^2 of *S*. Thompson biofilm on food contact surface. Single treatment of UV-C (1, 2 and 5 min) reduced 0.61 to 5.15, 0.80 to 4.74 log CFU/cm^2 of *S*. Typhimurium and *S*. Thompson biofilm on food contact surfaces, respectively. However, the biofilm on food contact surface was significantly decreased (P<0.05) by combination treatment of J.27 with UV-C. Combination treatment of J.27 with UV-C reduced 1.02 to 6.37 log CFU/cm^2 of *S*. Typhimurium biofilm and 1.65 to 6.38 log CFU/cm^2 of *S*. Thompson biofilm on food contact surface.

Significance: These results indicated that the synergistic treatment of UV-C assisted J.27 could be used for *Salmonella* biofilm control strategy in poultry industry.

P1-56 Interactions of *L. monocytogenes* with Non-Pathogenic *Listeria* Species in Biofilms and Transferring Capacity of Quaternary Ammonium Compounds Resistance Genes

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria* species including the pathogen *L. monocytogenes* are able to produce biofilms, which may lead to persistence inside food processing environments. In those structure the acquisition of resistance to disinfectants has been described, however, information about microbial interactions between *Listeria* species and gene transference inside biofilms is scarce.

Purpose: Microbial interaction between *L. monocytogenes* and non-pathogenic *Listeria* species inside biofilms was evaluated, in order to determine the potential transference of quaternary ammonium compounds (QACs) resistance genes among strains.

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Methods: Dual biofilms of *L. monocytogenes* ATCC 19115 and *L. grayi* (1), *L. innocua* (9), and *L. ivanovii* (3) containing *bcrABC* gene associated to QACs resistance were evaluated for gene transference. Stainless steel coupons were inoculated by immersion in tryptic soy broth and incubated at 35°C for 4 h. Coupons were washed, to removed unattached cells and keept at 35 °C for up to 10 days. Microbial populations (MP) and biofilm production (BP) were quantified by surface plating and crystal violet stain method, respectively. The transference of *bcrABC* gen from *Listeria* spp. isolates to *L. monocytogenes* ATCC 19115 was determinate by PCR detection.

Results: In dual cultures adhesion capacity of *L. monocytogenes* reduced in the presence of *L.* grayi, *L. innocua*, and *L. ivanovii* strains compared to pure cultures. At the end of storage, population of *L. monocytogenes* decreased in the presence of *L. innocua* (4/9), while non-antagonistic effect was observed with *L. grayi* and *L. ivanovii*. A similar pattern was observed with regards to BP. Transference of gen *bcrABC* was only observed in culture of *L. monocytogenes* with *L. innocua* (3/9 isolates).

Significance: The presence of *L. innocua* strains that contain *bcrABC* gene and show the capacity to transfer it to *L. monocytogenes*, represent a critical persistence risk of this pathogen inside food processing environments.

P1-57 Biofilm Formation Capacity and Disinfectants Resistance: Key Factor Involved in Persistence Risk of *Listeria monocytogenes* at Food Processing Environments

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Introduction: *Listeria* species including the pathogen *L. monocytogenes* can stablish at food processing environments. Biofilm formation capacity and disinfectants resistance such as quaternary ammonium compounds (QACs) influence their persistence at those environments.

Purpose: Characterize *Listeria* spp. strains based on biofilm formation capacity and presence of QACs resistance genes, to estimate the risk of persistence at food processing environments.

Methods: *Listeria* strains (119) isolated from food processing environment were identified by PCR-Multiplex. Biofilm production (BP) capacity of the strains was quantified measuring optical density at 35°C for 72 h according to crystal violet assay and classified as: none, weak, moderate, and strong. BP dynamics profiles were classified as: continuously increasing, increasing followed by decreasing, and null. Genes *qacH* and *bcrABC* were detected by PCR. Persistence risk of strains were estimated using a semi-quantitative scale (0-10) including BP and presence of QACs resistance genes.

Results: *L. monocytogenes*(75),*L. innocua* (20), *L. ivanovii* (10), *L. grayi* (7), *L. welshimeri* (4), and *L. seeligeri* (3) were identified. At 24 h the strains were classified as null (23), weak (63), moderate (32), and strong (1) biofilm producer, whereas at 72 h as null (32), weak (35), moderate (48), and strong (4). Continuously increasing BP dynamic was the most common (65%) followed by increasing with decreasing BP (28%), and null (7%). *bcrABC* gen was detected in *L. monocytogenes* (39/75), *L. innocua* (9/20), *L. ivanovii* (4/10), and *L. grayi* (3/7); *qacH* gen was not found. *L. grayi* and *L. ivanovii* showed highest persistence risk value (10) whereas the majority of *L. innocua* and *L. monocytogenes* strains presented values of 4 and 5, respectively.

Significance: According to the proposed semi-quantitative scale, *Listeria* strains displaying early biofilm release (BP increasing followed by decreasing dynamic) and carriers of *bcrABC* gene represent a higher persistence risk compared to those isolates that produce more stable biofilm structures.

P1-58 Variation in Resilience Phenotypes Among Sublineages of *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: *L. monocytogenes* has robust stress adaptive capabilities, which contribute to its ability to grow in diverse environments. The population structure of *L. monocytogenes* is divided into 4 lineages and many sublineages. Variation in resilience phenotypes, including the ability to grow under suboptimal conditions, exists among lineages of *L. monocytogenes*. Growth parameters under suboptimal conditions have yet to be compared extensively among sublineages of *L. monocytogenes*.

Purpose: To quantify growth parameters of strains representing the diversity of *L. monocytogenes* under multiple environmental stresses.

Methods: 317 *L. monocytogenes* strains were selected to represent the 4 lineages and 80 sublineages. Strains were grown in BHI broth at pH 5.5, pH 8.5, or with 4% and 7% NaCl and OD600 monitored over time in triplicate. Gompertz, Buchanan, and Baranyi models were used to fit growth parameters, and the Baranyi model was selected as it performed best among all strains and growth conditions. The average growth rates and lag phase were calculated for sublineages. Relationships between phenotype and sublineage were evaluated using analysis of variance ($p<0.05$).

Results: Significant differences in growth rate were identified among sublineages at 4% and 7% NaCl, and at pH 5.5. Significant differences were also identified in lag phase at 7% NaCl and pH 5.5. Compared to other sublineages (SL), SL87 and SL219 from lineage 1 and SL11 from lineage 2 had faster growth rates and shorter lag phases under these conditions, while SL9 and SL204 had relatively lower growth rates and longer lag phases. Among these sublineages, strains of SL9 are prevalent in foods, while SL87 are prevalent in farm environments.

Significance: Quantifying the stress adaptive capabilities among sublineages will allow for further modeling growth in more complex environments, such as food, and may explain the prevalence of certain subtypes in specific environments.

P1-59 Manifolds of Flavourzyme on Biofilm Formation, Quorum Sensing, and Virulence Gene Expression of *Pseudomonas aeruginosa*

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Introduction: The persistence of biofilms is a global burden on the food industry, as *Pseudomonas aeruginosa* is responsible for food degradation, posing a severe economic and public-health concern through cross-contamination.

Purpose: The goal of the study was to unveil the various impacts of Flavourzyme on *P. aeruginosa* biofilms and determine how it prevents the bacteria from forming biofilms and becoming hazardous at the molecular level.

Methods: The minimum inhibitory concentration (MIC), antibiofilm assessment by crystal violet and MBEC™ biofilm device assay, epifluorescence microscopic analysis, confocal laser scanning microscopic analysis, motility assay, butyryl homoserine lactone detection and verification with bioluminescence and high-performance liquid chromatographic assay, and genomic expression analysis by qRT-PCR were performed to determine diversified mechanisms of Flavourzyme against *P. aeruginosa*.

Results: Results revealed that a co-culture with 300 µl/ml (1 MIC) of Flavourzyme could kill *P. aeruginosa*. On the MBEC™ biofilm-forming device, more than 4.5 log CFU/peg biofilm was inhibited by 0.125 MIC of Flavourzyme. The motility and the production of 3-oxo-C_{12}-homoserine lactone and C_4-homoserine lactone were also reduced from 0.06 MIC and undetectable with 0.125 MIC of Flavourzyme. Interestingly, 0.03 MIC of Flavourzyme showed dramatic upregulations of quorum sensing and virulence regulating genes.

Significance: Based on our findings, we suggest specific concentrations of Flavourzyme against *P. aeruginosa* biofilm as an effective antibiofilm agent, presumably by blocking bacterial safeguarding machinery once the cellular proteins are disrupted. The findings will alert Flavourzyme users to use the product in specific doses to protect their food from *P. aeruginosa*.

Poster

P1-60 Survival of *Cronobacter sakazakii* on a Food Contact Surface at Refrigeration and Room Temperature

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Undergraduate Student Award Entrant

Introduction: *Cronobacter sakazakii* causes outbreaks resulting in high infant mortality rates, though infections can also occur in adults. The recent *C. sakazakii* outbreaks drive the need to better understand its survival characteristics to reduce transmission risk.

Purpose: The purpose of this study was to determine the survival of two strains of *C. sakazakii* (29544 and 29004) spiked in milk and spilled on stainless steel (SS) surfaces (model food contact surface) at room temperature (RT; 22.5°C) and refrigeration (4°C).

Methods: One-mL overnight cultures of *C. sakazakii* were aseptically mixed with 9 mL of UHT milk and 100 µL was aseptically spread on sterile 3x3 cm^2 SS coupons. The coupons were kept within sterile petri-plates and stored at 4°C or 22.5°C for up to one month and relative humidity (RH) was monitored. Bacteria were enumerated by surface-spread plating on Tryptic Soy Agar plates after incubation at 37°C for 24 h. Recovered counts from three replicate experiments plated in duplicate were statistically analyzed using JMP.

Results: At day 0, average *C. sakazakii* 29544 population levels were 7.79±0.29 log CFU/mL that were significantly (p≤0.05) decreased after day 1, 2, 5, 7, 9 and 30 to 6.72±0.15, 6.76±0.21, 6.46±0.37, 6.20±0.33, 5.85±0.43 and 5.61±0.69 log CFU/mL, respectively at RT (22.5°C, 52.9% RH). At 4°C (82.2% RH), population levels were 7.93±0.32 log CFU/mL on day 0, with lower decreases to 7.88±0.25, 7.74±0.26, 7.32±0.22, 7.26±0.45, 7.39±0.51 and 6.97±0.29 log CFU/mL after day 1, 2, 5, 7, 9 and 30, respectively. Similar trends were observed for *C. sakazakii* 29004.

Significance: This study provides data for risk assessment analysis that show increased survival of *C. sakazakii* during a contamination event at 4°C and high humidity than at 22.5°C and lower humidity over 30 days. Thus, there is an increased risk of *C. sakazakii* cross-contamination via a contaminated contact surface at lower temperatures.

P1-61 Evaluation of Longer-Term Biofilm Formation of *Listeria monocytogenes* Strains Influenced by Media Compositions

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Introduction: Biofilm is a major reservoir of persistent contamination by *Listeria monocytogenes*. Biofilm formation is influenced by bacterial cell physiology and abiotic conditions such as temperature, nutrient composition, and surface properties.

Purpose: Most studies on *L. monocytogenes* biofilm were conducted in a 1- to 2-day timeframe. Here, we evaluated the effect of common growth media on 3- and 10-day biofilm formation by a wide selection of *L. monocytogenes* isolates from clinical, food, and environmental sources.

Methods: We tested 27 *L. monocytogenes* strains in LB (1% NaCl), BHI, TSB, MH and 1/10x LB and BHI to evaluate biofilm formation (crystal violet binding) and cell concentration (enumerated on 6x6 drop plates) at 30°C, 3 and 10 days post-inoculation. Additionally, LB-no salt (LBNS) and BHI + 1% NaCl (BHIS) were tested to assess the influence of NaCl. Media pH and dissolved oxygen levels were determined using a microbioreactor. Experiments were repeated at least twice on different days.

Results: Biofilm formation was strongly strain-dependent. Some media favored biofilm formation: >70% of strains formed at least moderate biofilm in LB, >50% formed moderate biofilm in BHIS. BHI, TSB, and LBNS were the worst media for biofilm formation. NaCl levels ≥1% favored biofilm formation. One-tenth-strength LB or BHI resulted in poor growth and poor biofilm formation. Poor biofilm formation in TSB might be explained by a more dramatic drop in media pH.

Significance: *L. monocytogenes* biofilms can persist in factories for decades. Laboratory studies of *L. monocytogenes* biofilms help food producers to make science-based decisions. TSB is commonly used for *L. monocytogenes* biofilm assays but, in comparison to other media, poorly supports biofilm formation. This may have led to an underestimate the ability of *L. monocytogenes* to form biofilm in previous reports. Our findings should help improve future experimental design to evaluate *L. monocytogenes* capability in biofilm formation.

P1-62 Isolation of Different Colony Morphotypes of *Listeria monocytogenes* after Exposure to High and Low Concentrations of First Generation Qac Benzalkonium Chloride (BAC) in Water

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Introduction: Despite the modernization of regulations and sanitization procedures, outbreaks of *Listeria monocytogenes* have been on the rise for the last 20 years. The increasing prevalence of *L. monocytogenes* requires an understanding of the factors that allow it to survive in food processing environments. One of the factors could be a growing tolerance to the most widely used disinfectant benzalkonium chloride (BAC) and if any morphotypes are formed.

Purpose: The objective of this study was to investigate the BAC tolerance of colony morphotypes of *L. monocytogenes* relative to their parent strain.

Methods: The morphotype variants of *L. monocytogenes* obtained after a two-step sequence of lethal (10-14 µg/ml for 1 h in water) and sublethal (2.5 to 7 µg/ml for 24 h in broth) exposure were tested against BAC for increased tolerance if accompanied by morphotype variation. The irregular colony variants recovered on BAC containing agar (6-8 µg/ml) were tested against the parent strain in a 3 h time-to-kill (TTK) assay with lethal BAC in water.

Results: Of the 48 morphotype variants initially collected on BAC containing agar, 35 morphotypes survived the initial screening. Eight variants, representing four of each Bug600 and ScottA were selected that broke the MIC of the parent strain in broth, and at least doubled the MIC on agar. Morphotypes were then compared against the parent strain in a 3 h TTK assay at lethal BAC (12 or 14 µg/ml). The parent strain could not be recovered past 1 h, whereas morphotypes survived for 3 h by 1 to 3 log CFU/ml. Finally, a repeat of the initial MIC experiment revealed a 2 to 3-fold increase in MIC of the morphotype variants.

Significance: These findings show that repeated exposure to low levels of BAC will result in some levels of BAC tolerance among *L. monocytogenes* that might be mediated by morphotype variation.

P1-63 Growth, Virulence, and Global Gene Expressions of Foodborne *E. coli* O157:H7 in the Presence of Microplastics and Nanoplastics

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Introduction: Microplastics/nanoplastics (MNPs) are emerging pollutants in the ecosystem capable of altering microbial physiology and activities. Interaction with MNPs may change the gene expression of foodborne microbes, affecting their growth and pathogenicity. However, the information on MNP-foodborne pathogen interactions is limited. To address this, we evaluated the growth pattern, gene expression of *E. coli* O157:H7 upon exposure to different types of polystyrene (PS) microplastics (MPs) and nanoplastics (NPs).

Purpose: Our study aimed to explore the influence of MNPs on the growth and changes in the gene expression of *E. coli* O157:H7 EDL933.

Methods: Growth of *E. coli* was studied for 24h in LB broth and compared for changes upon exposure to surface-charged^{+ve}/$^{-ve}$ PS-NPs (30-50 nm) and PS-MPs (0.9-1.1 µm), at 10, 50 and 100 mg/l concentrations. The global gene expression was evaluated by RNA-Seq using Salmon's decoy-aware, followed by one-way ANOVA with pairwise comparisons for multiple testing using the False Discovery Rate method. The relative gene expression was computed

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using real-time PCR, targeting virulence (*stx1A*, *stx2A*, *eaeA*, *fliC*, *bolA*), stress-response (*rpoS, uspB, chpB*, *rpoH)* and biofilm formation genes *(hipA, luxS*) upon exposure to MNPs (24h). One-way ANOVA was performed to find the statistical significance of means of three trials of each treatment (in triplicate).

Results: Modified-Gompertz growth model revealed a longer generation time (65 min) of *E. coli* upon exposure to PS-NPs^{+ve} compared to the control (28 min). A total of 2119 genes were found to be differentially expressed upon MNP exposures. Downregulation of *rpoS* was observed for all MNPs. Significant (P<0.001) upregulation of *fliC* ($log^{Fold\text{-}change}$ 2.65), while down-regulation of *rpoH* ($log^{Fold\text{-}change}$ 2.7) and *rpoS* ($log^{Fold\text{-}change}$ 3.03) were observed for MPs^{+ve} exposure. Downregulation of *hipA* ($log^{Fold\text{-}change}$ 3.3), while upregulation of *luxS* ($log^{Fold\text{-}change}$ 2.46) were observed for MNPs^{+ve}.

Significance: This study sheds light on the physiological impact of MNP-exposures to an important foodborne pathogen.

P1-64 Synergistic Effects of Disinfectants with E-Beam for Inactivation of Hepatitis A Virus on Fresh Vegetables

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Introduction: Hepatitis A virus (HAV) has adversely affected public health worldwide. Fresh vegetables are commonly reported as the source of HAV infections, which in turn, causes foodborne illnesses. Therefore, chemical, and physical sequential treatment can be applied for HAV inactivation on fresh vegetables.

Purpose: This study investigated the synergistic effects of disinfectant including sodium hypochlorite (NaOCl) and chlorine dioxide (ClO_2) with electron beam (e-beam) on fresh vegetable (bell pepper and cucumber) contaminated with HAV.

Methods: Each disinfectant of NaOCl (0-500 ppm for bell pepper; 0-200 ppm for cucumber) and ClO_2 (0-250 ppm for bell pepper; 0-40 ppm for cucumber) with e-beam (0-5 kGy for bell pepper; 0-1.5 kGy for cucumber) were sequentially treated on each sample. The viral titer was measured by $TCID_{50}$ assay after recovery.

Results: The combined treatment of 500 ppm NaOCl with 3 kGy e-beam on bell pepper and 150 ppm NaOCl with 1 kGy e-beam on cucumber provided maximum synergistic effect. In addition, 50 ppm of ClO_2 with 5 kGy of e-beam on bell pepper and 10 ppm ClO_2 with 1.5 kGy of e-beam on cucumber were the most efficient methods for reducing the vegetables.

Significance: This study suggests that the sequential treatment of NaOCl and ClO_2 with e-beam can effectively control the HAV on fresh vegetables.

P1-65 Preventive Effect of Glucose Oxidase and Potassium Sorbate Singly and Combined Against *E.coli* Biofilm on Food and Food Contact Surfaces

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Introduction: *Escherichia coli* can cause global food-borne illness and is well known as a bacterium that threatens public health. In addition, its biofilm is usually found in various kinds of foods, so several experts are focusing on reducing *E. coli* biofilm.

Purpose: The purpose of this study was to investigate the antibiofilm activity of glucose oxidase and potassium sorbate singly and combined.

Methods: The *Escherichia coli* O157:H7 strain and culture were prepared, and MICs of glucose oxidase and potassium sorbate against *E. coli* were determined. *E. coli* was allowed to develop biofilm on biotic (shrimp, beef) and abiotic (PET, rubber) surfaces and treated with different concentrations of glucose oxidase and potassium sorbate. After treatment, detachment and CLSM and FE-SEM were conducted to visually confirm the antibiofilm ability of glucose oxidase and potassium sorbate.

Results: The single treatment of glucose oxidase and potassium sorbate reduced approximately 2.5, 2.3 log CFU/g and 3.2, 2.9 log CFU/g *E. coli* biofilm at 4 MIC from shrimp and beef, respectively. In another other case, 2.5, 2.6 log CFU/cm^2 and 2.2, 2.3 log CFU/cm^2 reduced at 4 MIC PET and rubber, respectively. In the combination treatment, the reduction rate was approximately 4.0 and 3.5 log CFU/g, 3.0 and 3.10 log CFU/cm^2 *E. coli* biofilm at 4 MIC from shrimp, beef, PET, and rubber, respectively. The reduction effect of the combined treatment of glucose oxidase and potassium sorbate against the biofilms of *E. coli* was also visually confirmed by confocal laser scanning microscopy (CLSM) and field emission scanning electron microscopy (FE-SEM).

Significance: This study confirmed that the combination treatment of glucose oxidase and potassium sorbate was effective in reducing *E. coli* biofilms and increases the food safety of the food industry.

P1-66 Alternative Rapid Method to Enumerate Yeast and Mold in Low-pH Foods

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Introduction: Low-pH foods are susceptible to fungi which are often acid tolerant and prone to cause quality issues. The traditional method, GB 4789.15-2016 for yeast and mold enumeration in food samples, uses Rose Bengal Medium and requires up to 5 days of incubation. Early observation of the plates is required after 3 days to minimize interference from spreading colonies. Alternatively, a rapid method is available which reduces the incubation time to 48 hours and controls the spreading of mold colonies.

Purpose: This study aimed to compare the performance of an alternative rapid method to GB 4789.15-2016 for mold and yeast enumeration in low pH foods.

Methods: Four low pH food matrices (catsup, tomato sauce, mayonnaise, and salad dressing) were artificially contaminated with *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii* and *Aspergillus niger*, respectively. Each organism spiked at high (~10^6 CFU/g), medium (~10^4 CFU/g) and low (~10^2 CFU/g) levels (4*3*3 contaminated samples). The total yeast and mold count in each contaminated sample was determined following the rapid alternative method using 3M Petrifilm Rapid Yeast and Mold Count Plates incubated at 28°C for 48 hours (n=5) and GB 4879.15-2016 (n=5).

Results: The mean log difference between the rapid alternative method and GB 4789.15-2016 among all samples was within ± 0.5 log_{10}. Relative trueness analyses showed a bias of 0.035, a 95% upper limit of 0.203 and a 95% lower limit of -0.134. The accuracy profile study showed that bias among all tests were within their β-expectation tolerance intervals.

Significance: The rapid alternative method demonstrated accurate and repeatable results in 48 hours and was comparable method to the traditional agar-based method for enumeration of yeast and mold in low-pH foods. The reduction in incubation time can save food factories 3 days of additional incubation and help free up storage space.

P1-67 Carbon Utilization Variances of *Campylobacter jejuni* strains Associated with Two Different Clinical Manifestations

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Introduction: *Campylobacter jejuni* produces two different diarrheal outcomes in humans: a bloody/inflammatory diarrhea or a watery diarrhea. Currently, little is known about the factors involved in these clinical manifestation differences. Previous results from our laboratory demonstrated strains associated with watery diarrhea are poor/non-invasive, whereas bloody/inflammatory diarrhea-associated strains are invasive, suggesting an extracellular versus intracellular lifestyle in humans, respectively.

Purpose: We hypothesize that *C. jejuni* carbon utilization within the host has a critical role in the variation of diarrheal development between strains. Therefore, in this study, we investigated the utilization of different carbon sources between five *C. jejuni* strains associated with bloody/inflammatory diarrhea and five associated with watery diarrhea.

Methods: Initially, all ten strains were screened via phenotype microarray for the utilization of 192 different carbon sources with fucose and glutamine utilization identified as different between the groups. Next, sole carbon growth curve assays at 37°C (human) and/or 42°C (poultry) for mucin, fucose (component of mucin), and glutamine were done to confirm group differences and compare different host temperatures.

Results: We found at 37°C there was a statistically significant higher utilization of mucin (p=4.699e-07) and fucose (p=2.265e-15) by the watery diarrhea strains compared to the bloody/inflammatory diarrhea strains. While there was a significantly higher utilization of glutamine (p=1.483e-09) at 37°C by strains associated with bloody/inflammatory diarrhea compared to watery diarrhea strains. However, at 42°C the only significant difference was an increased utilization of fucose (p=1.721e-07) by strains associated with watery diarrhea.

Significance: Overall, our results further the understanding that poor/non-invasive *C. jejuni* strains associated with watery diarrhea have adapted an extracellular lifestyle using fucose and mucin as a carbon source. Whereas invasive strains associated with bloody/inflammatory diarrhea use glutamine as a carbon source, which is an amino acid associated with tight junction integrity for enterocytes.

P1-68 Beneficial, Safety and Antioxidant Properties of Potential Probiotics Lactic Acid Bacteria

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Introduction: The role of lactic acid bacteria (LAB) as beneficial cultures as health promoting factors has gained increasing interest during the last decades. This is reflected in screening of LAB strains in line with minimal requirements for a “probiotic” with regard to safety and functionality, include natural antioxidant properties, thereby constituting an additional benefit in substituting synthetic antioxidants.

Purpose: Based on the *in vitro* safety and antioxidant assays conducted in this study to select functional and safe strains for further applications as beneficial cultures (probiotics and/or starter cultures).

Methods: *Lactobacillus gasseri* ST16HK, *Streptococcus salivarius* ST48HK, ST59HK, ST61HK, ST62HK, *Lactiplantibacillus plantarum* ST63HK, ST66HK, *Latilactobacillus sakei* ST69HK, *Enterococcus faecium* ST651ea, ST7119ea, ST7319ea previously evaluated as potential probiotics were assessed regarding their safety and antioxidant properties.

Results: Studied strains shown to be γ-hemolytic, not producers of biogenic amines, lipase and protease negative, without mucin degradation properties. Analysis of DPPH free radical-scavenging property of microorganisms showed ST59HK to exhibit the highest activity at a level of 85.24%. The highest Fe^{+2} chelation activity with 98.2% was observed for ST62HK while the lowest was recorded for ST48HK at 71.5%. The highest and lowest hydroxyl radical scavenging levels were detected for ST59HK (98.6%) and ST63HK (35.60%), respectively. Superoxide anion radical scavenging activity was highly exhibited by ST61HK (54.62%) and the least exhibited by ST651ea (18.7%). Lastly, the strains ST16HK and ST7319ea showed the highest and lowest anti-lipid peroxidation levels with 69.43% and 26.15%, respectively.

Significance: Concept of “safety first” need to be considered as researchers’ responsibility and priority and combined with evaluation of the specific beneficial properties can be regarded as initial step in the selection of putative probiotic strains. Anti-oxidative properties appear to be strain specific and thus, some of these strains could be potentially applied as natural antioxidants in fermented food products.

P1-69 Viability of Probiotics Incorporated in Edible Coatings Added of Fructooligosaccharides to Preserve Fresh-Cut Mango and Melon

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Introduction: Probiotics are incorporated into foods as functional ingredients. Fructooligosaccharides (FOS) are metabolized by probiotics, increasing their viability in foods. Fresh-cut fruits are practical, healthy foods that attract consumers’ attention, and edible coatings can extend their short life. The impacts of FOS addition on the viability of probiotics incorporated in coatings of fresh-cut fruits remains unknown.

Purpose: To evaluate the survival of *Lacticaseibacillus casei* 01 (Lc-1) incorporated in alginate coatings added of FOS applied to fresh-cut mango and melon.

Methods: Coatings were composed of 1.5% alginate (w/v), 0.75% glycerol (v/v), 0.04% sunflower oil (v/v), 0.05% tween 80 (v/v), and sterile distilled water. Lc-01 (7 log CFU/ml) and 1.5% FOS (w/v) were incorporated into the coating solution. Coatings without FOS were evaluated as control. Mango and melon cubes (6cm3) were immersed (2 min) into the coating solution, followed by (1 min) the crosslink solution (2%CaCl2; w/v). Samples were stored at 5°C and analyzed at 1, 3, 6, 9, and 12 days. Lc-1 was enumerated by plating on MRS-vancomycin (0.05 mg/mL). pH, titratable acidity (TA), and total soluble solids (TSS) were measured. Three independent batches were performed. All analyses were done in triplicate.

Results: Lc-01 decreased 1 log CFU/g after three days in mango coated without FOS but did not differ from initial counts after 12 days. In melon, Lc-1 counts decreased 1 log CFU/g after nine days without further changes. Lc-1 counts did not change in mango or melon FOS-coated over the storage. pH increased in mango without FOS after three days, while it did not change in FOS-coated mango. In melon, the pH increased over the storage, particularly in FOS-coated melon. TA decreased, and TSS increased, regardless of the coating.

Significance: The results show that FOS maintains the viability of Lc-01 in coatings without compromising the fruit quality parameters but fruit type impacts the probiotic behavior.

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P1-70 Growth Potential of *Bacillus cereus* Group Strains from Different Phylogenetic Groups in a Dairy Food Model

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❖ ***Developing Scientist Entrant***

Introduction: The *Bacillus cereus* group is comprised of eight valid genomospecies distributed among eight phylogenetic groups. Some *B. cereus* group strains can cause disease or food spoilage. Strains that express virulence factors, including enterotoxins, are cytotoxic toward human gut epithelial cells when they grow to a high concentration. The ability of strains to grow to a high concentration in food is a factor that needs to be considered in exposure assessments.

Purpose: This project aimed to assess potential differences in the growth potential of 17 *B. cereus* group strains of different genotypes in a dairy food model.

Methods: Spores of 17 cytotoxic *B. cereus* group isolates from six phylogenetic groups and different virulotypes were used to determine their growth rates in a skim milk broth. Strains were inoculated in a skim milk broth at ~10^3 spores/ml and incubated at 4°C, 10°C, and 22°C for 504, 504, and 32 hours, respectively. Bacterial numbers were quantified by spiral plating onto brain heart infusion agar with subsequent incubation at 30°C for 12 to 16 hours. Colonies were counted using an automatic plate counter. The R package Growthcurver was used to fit the growth curves. The growth of isolates at different temperatures was compared using a one-way ANOVA with a Tukey post-hoc test.

Results: Isolates did not grow at 4°C. All but two isolates grew at 10°C, while all strains grew at 22°C. There was a significant difference in the estimated

maximum population size, but no significant difference in the maximum growth rate of isolates grown at 10°C compared to those grown at 22°C. Lastly, the group I isolates grew significantly less at 22°C (*P*=0.00139) and 10°C ($P=7.76*10^{-7}$) when compared to isolates from all other groups.

Significance: The observed differences in the growth rates of *B. cereus* group isolate among phylogenetic groups demonstrate the need for a phylogenetic group-based exposure assessment.

P1-71 Street Food as a Reservoir for Colistin-Resistant and Extended-Spectrum β-Lactamase (ESBL)-Producing *E. coli* and *Klebsiella* spp. in Bangladesh

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❖ ***Developing Scientist Entrant***

Introduction: Antibiotic-resistant pathogens disseminated through street foods are of major public health concern since a significant proportion of the urban population in developing countries consumes street foods regularly.
Purpose: This study aimed to determine the prevalence of colistin-resistant and ESBL-producing *E. coli* and *Klebsiella* spp. in street foods in Sylhet city, Bangladesh, and evaluate their antibiotic susceptibility pattern.
Methods: One hundred fifty samples of street foods (phuchka, n=45; chatpati, n=35; bhelpuri, n=25; chola, n=20; salad, n=25) were collected from randomly selected street vendors and screened for coliform bacteria. Seventy-five *E. coli* and 25 *Klebsiella* spp. were isolated and identified by the IMViC test. The isolates were tested for their susceptibility to colistin by measuring the MIC using broth microdilution method. Antibiotic susceptibility of the colistin-resistant isolates was investigated by the Kirby-Bauer disk diffusion method. The ESBL production was examined using the Double Disc Synergy Test (DDST) and Phenotypic Confirmatory Disk Diffusion Test (PCDDT).
Results: Thirty-five (46.67%) *E. coli* and nine (36%) *Klebsiella* spp. isolates with MIC values ≥4µg/ml were confirmed as colistin-resistant per the Clinical and Laboratory Standard Institute (CLSI) guidelines. All of the colistin-resistant isolates showed multidrug resistance (MAR≥2), and 56.82% (n=25) of the isolates were found to be resistant to at least one of the third- and fourth-generation cephalosporins (ceftazidime, ceftriaxone, and cefepime) tested. Resistance and sensitivity of 18 antibiotics were significant for *E. coli* (p<0.05). *E. coli* isolates were sensitive to aztreonam, fosfomycin, and kanamycin, while *Klebsiella* spp. isolates were sensitive to chloramphenicol and vancomycin. ESBL was detected in 11 (31.43%) *E. coli* and 3 (33.33%) *Klebsiella* spp. by DDST and in 8 (22.86%) *E. coli* and 2 (22.22%) *Klebsiella* spp. by PCDDT.
Significance: This study illustrates the growing resistance of food-borne pathogens against the last-resort antibiotic colistin and encourages further research on the safety of street foods in Bangladesh.

P1-72 Cross-Contamination of High Touch Kitchen Surfaces during Breakfast Meal Preparation

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Introduction: Consumer home meal preparation remains a significant contributor to foodborne illness. Understanding cross-contamination to kitchen surfaces during home meal preparation is important for the design of targeted risk reduction strategies.
Purpose: Quantify cross-contamination to representative surfaces during consumer preparation of a breakfast meal.
Methods: A 1-lb chub of ground breakfast sausage was inoculated with 10^9-10^{10} CFU GFP-tagged *Escherichia coli* DH5-α. A meal consisting of ground sausage, eggs, and a salad containing cantaloupe was prepared by participants (*n*=125) in test kitchens (IRB protocol:10599). Environmental and cantaloupe samples were collected and screened for *E. coli* contamination using modified Tryptic Soy Agar.
Results: The highest prevalence of contamination corresponded to the sink basin (*n*=43/125, 34.4%) and the cantaloupe (*n*=33/125, 26.4%) which had significantly higher contamination prevalence compared to all other surfaces (*p*<0.005). Spice containers (*n*=8/125, 6.4%), juice glasses (*n*=7/125, 5.6%), and the recipe 'tablet' (*n*=5/125, 4.0%) were contaminated at lower frequencies. Overall, *E. coli* concentrations were relatively low (1-4.6 $\log_{10}$ CFU/sample), with the highest concentrations in sink basin samples (>3-$\log_{10}$ CFU/100 cm^2 for 25% of samples) followed by cantaloupe [>2.3 $\log_{10}$ CFU/25g with 10% >3.5 $\log_{10}$ CFU/25g]. There were no statistically significant differences between mean *E. coli* concentrations when comparing samples (*p*=0.181).
Significance: This study provided information on prevalence and degree of cross-contamination from raw sausage to representative kitchen surfaces during breakfast meal preparation. Further studies investigating specific behaviors or actions that lead to cross-contamination are warranted. This information could be used to inform risk management practices to reduce the likelihood of cross-contamination during home meal preparation.

P1-73 *Salmonella* Serotypes Uncommonly Found in FDA-Regulated Food Commodities

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Introduction: Food commodities are the main source of *Salmonella* infections in human illnesses. Common outbreak strains are well studied but less is known about rare *Salmonella* serotypes that are repeatedly found in certain food commodities. It is important to understand the complexity of different *Salmonella* serotypes and why some serotypes are less common than others.
Purpose: The purpose of this study was to analyze uncommon *Salmonella* serotypes found in various FDA regulated food commodities such as ground melon seeds, sesame seeds, and papaya.
Methods: NCBI Pathogen Detection was used to survey uncommon *Salmonella* isolates from various foods commodities, and GalaxyTrakr was used to characterize genetic differences between serotypes.
Results: Twenty-two (22) *Salmonella* serotypes were found associated with ground melon seeds (also known as Egusi) which included some rare serotypes such as Colobane, Gbadago, Tokoin, Jodhpur, Ealing, Benue, Bousso and Lowestoft. We will describe other food commodities found to be contaminated with uncommon *Salmonella* serotypes and investigate genetic differences between these rare serotypes and common outbreak strains. Genetic characterization will include AMR and virulence genotypes, phylogenetic clusters, and SNP analysis.
Significance: This study shows that there are rare *Salmonella* serotypes that can be found in various food commodities. Investigations into these rare serotypes may provide some explanation as to their origin, pathogenicity and prevalence in certain foods. Further research needs to be performed to compare genotypic and phenotypic differences that may explain the differences in prevalence of certain uncommon *Salmonella* serotypes in foods.

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P1-74 Antimicrobial Activity of Nanoemulsified Benzyl Isothiocyanate Against *Escherichia coli* O157:H7 during Storage

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Introduction: Consumers' preference for minimal chemicals in food has led research on natural antimicrobials and their nanoemulsion.

Purpose: To synthesize Benzyl Isothiocyanate nanoemulsion (BITNM) and determine its antimicrobial activity against *Escherichia coli* O157:H7 (EHEC) during storage.

Methods: The oil-in-water BITNM 0.5% was prepared using monolaurin, ethanol, tween 80 and spam 80. The droplet size of BITNM was determined by using Zetasizer. BITNM was stored at three temperatures (4, 25 and 37°C) for 56 days. On days 0, 7, 14, 28, and 56, the antimicrobial activity of BITNM against five EHEC strains was determined by well diffusion assay. Briefly, individual EHEC culture (6 log CFU/ml) was streaked on the tryptic soy agar plates and air-dried in the biosafety hood. After drying, 100 µl of BITNM or pure BIT (0.5%) in sterile water was added to each well of the plates. The plates were incubated at 37°C for 24 h and the inhibition zones (mm) were measured. Each experiment was repeated twice with triplicate samples (N=450). Data were analyzed by PROC-Glimmix of SAS and the differences were detected at P<0.05.

Results: BITNM exerted higher antimicrobial activity against all EHEC strains resulting in inhibition zones of 13.4 to 18.5 mm dia. as compared to pure BIT at 8.1 to 12.7 mm on day 0 ($P<0.05$). On day seven, the antimicrobial activity of BITNM was significantly higher than the pure BIT regardless the storage temperature. BITNM stored at 4°C remained most effective against EHEC during storage; up to 15.4 mm of inhibition zones were measured on day 56. The droplet size of freshly made BITNM was 15.7 nm and remained stable for 56 days when stored at 4°C .

Significance: Results reveal the enhanced antimicrobial efficacy of BITNM *in vitro*. The water-soluble BITNM could be used as an antimicrobial treatment in variety of applications to improve food safety.

P1-75 Evaluation of Food Safety of Homemade Fermented Foods

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Introduction: Recently, the consumption of locally made traditional fermented foods along with dairy fermented products have been increasing in South Korea. However, there is limited information of the public health risk of microorganisms in locally made fermented foods.

Purpose: The purpose of this study was to evaluate the food safety of locally homemade fermented foods.

Methods: The homemade fermented food samples, including soybean paste (Korean traditional fermented food), cream cheese and yogurt were collected from the traditional local markets. The samples were cultured in different selective mediums (MRS agar, modified RCM agar, M17 agar, and Enterococcosel agar) with different culture conditions. The isolated colonies were then identified using the full-length 16S rRNA gene sequencing through Sanger sequencing. Molecular Evolutionary Genetics Analysis (MEGA) software were used for 16S rRNA gene sequence analysis. Identified bacterial species was classified based on the criteria of the GRAS (Generally Recognizes As Safe, FDA) and QPS (Qualified Presumption of Safety, EFSA).

Results: A total of 270 bacterial colonies were isolated, and a total of 74 probiotics strains were identified. Isolated bacterial species in the soybean paste included *Enterococcus faecium*, *Bacillus amyloliquefaciens*, *B. velezensis*, and *B. subtilis*. In cream cheese, *Streptococcus salivarius*, *E. faecalis*, *Lactobacillus acidophilus*, *E. faecium*, and *B. licheniformis* were identified. However, only three bacterial species including *Leuconostoc mesenteroides*, *S. thermophilus*, and *E. faecalis* were identified in yogurt. No pathogenic bacteria were identified.

Significance: Our data provide the basic information on bacterial species in homemade fermented foods in South Korea. Overall, our data suggested that the homemade fermented foods can be safely prepared and consumed.

P1-76 Activity of *Β*-Glucuronidase, Harmful Enzyme, in Lactic Acid and Foodborne Pathogenic Bacteria Isolated from Food and Infant Feces

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Introduction: *β*-glucuronidase is an enzyme that can induce colorectal cancer by hydrolyzing carcinogenic glucuronide. The activity of *β*-glucuronidase is determined by bacteria and the intestinal environment. *Clostridium*, *Bacteroides*, and *Eubacterium* are well known bacteria that produce *β*-glucuronidase. However, recently it has been reported that lactic acid bacteria also produce *β*-glucuronidase. Therefore, it is necessary to confirm whether lactic acid bacteria used as major probiotic microorganisms produce *β*-glucuronidase for the safety of administration.

Purpose: This study aims to examine the activity of *β*-glucuronidase in bacteria isolated from food and infant feces.

Methods: A total of 240 bacteria isolated from kimchi, cheese, beef, and infant feces was examined to confirm the activity of *β*-glucuronidase. Forty microliters of *p*-nitrophenyl-*β*-D-glucuronide, 760 µL of PBS, and 200 µL of bacteria inoculum were mixed and reacted at 37°C for 8 h. After adding 1 mL of 0.5 N NaOH to each sample and centrifuging at 3,000×g for 10 min, the supernatant was taken into a new tube. The absorbance of each sample was then measured at 405 nm, and *Escherichia coli* KCTC1682 was used as a positive control.

Results: Of the 119 samples, a total 240 strains were isolated including 68 strains of lactic acid bacteria. 57 strains were isolated from infant feces, 87 strains from kimchi, 12 strains from cheese, and 84 strains from beef. Among 240 isolates, *Shigella sonnei* HW66-1 strain isolated from beef had the highest *β*-glucuronidase activity, at 0.049 µM/mL/min. Among 68 strains of the isolated lactic acid bacteria, the highest *β*-glucuronidase activity strains were *Lactobacillus fermentum* SMFM2016-NK1 (0.0057 µM/mL/min) and *Lactobacillus gasseri* SMFM2021-S8 (0.0055 µM/mL/min).

Significance: This study indicates that the lactic acid bacteria and foodborne pathogenic bacteria isolated from food and infant feces could have *β*-glucuronidase activity, which could be associated with the development of colorectal cancer.

P1-77 Sequential Fermentation of Grape Must Using *Saccharomyces* and *Non-Saccharomyces* Yeasts

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Introduction: indigenous yeasts involved in the fermentation process influence alcohol content, pH, viscosity, color, the concentrations of sulfur compounds, phenolic compounds and volatile metabolites with the potential for strong influences on specific flavor and aroma of the final product. Therefore, it is important to study fermentation techniques and properties of indigenous yeasts to improve wine quality.

Purpose: the objectives of this study were to characterize and select indigenous yeasts for wine fermentation, and conduct sequential fermentation using *Saccharomyces* and non-*Saccharomyces* yeasts to determine wine quality attributes.

Methods: yeasts were characterized through testing their alcohol and SO_2 tolerance, β-glucosidase activity and H_2S production. *Saccharomyces uvarum* isolates *114256-2, 12246-2, 14233-2,* and *non-saccharomyces* isolates *Hanseniaspora uvarum, Wickerhamomyces anomalus* and *Zygotorulaspora florentina* were selected for sequential fermentation trials. Sauvignon Blanc concentrate was used as the base of the must, and *Saccharomyces cerevisiae* EC-1118 was used as the control. Three biological replications of the inoculum for each isolate and the control were prepared. One liter media bottle filled with the must was used for each experimental unit and each inoculated bottle was fitted with a fermentation lock and placed in a fermentation room at 23˚C for 24 days.

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S. cerevisiae EC-1118 was sequentially inoculated to the samples at two different times during fermentation. There were 44 fermentation samples for the trials.

Results: yeasts were selected based on their ethanol tolerance, free SO_2 tolerance>20mg/L, β-glucosidase activity positive and low H_2S production. After sequential fermentation using *S. uvarum*, the alcohol levels, reducing sugars and total acidity in samples ranged from 8.0-10.7%, 59.2-108.2g/L, 5.4-5.8g/L compared to 11.3±0.1%, 48.7±1.3g/L, 6.0±0.1g/L in the control, respectively. After fermentation using non-*Saccharomyces* yeasts, they ranged from 6.0-10.1%, 60.5-134.1g/L, 5.6-6.0g/L compared to 10.8±0.1%, 54.0±3.6g/L, 6.1±0.02g/L in the control, respectively.

Significance: the research results provide useful information on sequential fermentation and utilizing indigenous yeast strains in wine fermentation.

P1-78 Isolation and Characterization of Lactic Acid Bacteria from Kimchi for Antimicrobial Activity and Acid Tolerance as Possible Probiotics

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Introduction: Kimch is a traditional Korean food made of fermenting cabbages with various beneficial lactic acid bacteria (LAB). Probiotics are microbial feed additives that improve intestinal microbial balance in animals and humans. The efforts to develop new efficacious probiotics are currently increasing.

Purpose: The aim of current study was to screen and identify novel LAB strains as potential probiotics.

Methods: LAB were isolated from homemade and commercial kimchi purchased in traditional markets using MRS agars and identified by 16S rRNA sequencing analyses. The LAB strains were tested antimicrobial activities for E. coli, Staphylococcus aureus, Salmonella enteritidis, and S. typhimurium by spot-on-lawn method using live LAB and agar-gel-diffusion assay using culture supernatant. Acid tolerance tests were conducted by incubating LAB in pH 2.5 MRS broth for 2 h and 4 h considering the normal transit time through the stomach.

Results: A total of 111 LAB strains were isolated from Kimch including Lactobacillus sakei (n=75), L. plantarum (n=18), L. curvatus (n=11), L. paracasei (n=3), L. rhamnosus (n=1), L. paraplantarum (n=1), L. brevis (n=1), and L. acidophilus (n=1). Among them, 85 LAB strains showed antimicrobial activity against at least one pathogen. In the acid tolerance test, 18 LAB strains showed more than 95% survival rate both after 2 and 4 h incubation in pH 2.5 solution. Taken together, 5 new LAB strains (four L. plantarum and one L. sakei) were identified as novel probiotic candidates with great antimicrobial activity against all four pathogenic bacteria and high acid tolerance characteristics.

Significance: More than 100 LAB strains were newly isolated from Kimchi and screened for probiotic properties. Four L. plantarum and one L. sakei were chosen as new probiotic candidates based on antimicrobial function and potential to reach to intestine. Further in vivo studies may need to demonstrate their probiotic function.

P1-79 Synergistic Effect of Sequential Treatment with 222 Nm, 280 Nm, and 405 Nm Light Wavelengths on Inactivation of Foodborne Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: Light technologies with different wavelengths can mitigate the risk of contamination, but each wavelength range has limitations. A hurdle system consisting of sequential wavelengths treatments may enhance the disinfection performance by different mechanisms. This research evaluates the effectiveness, mechanisms of inactivation, and kinetics of sequential treatments with far-UVC (222 nm)+visible light (405 nm) and UV-C (280 nm)+visible light (405 nm) against several foodborne pathogens.

Purpose: This study evaluated the effect of far-UV-C, UV-C, and blue LED sequential light treatments against several major foodborne pathogens and evaluated their inactivation mechanisms and kinetics.

Methods: Early stationary phase (18h) colonies of *Escherichia coli* O157:H7, *Listeria monocytogenes*, Salmonella Typhimurium, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were inoculated in thin liquid films (1.2 mm thickness), and exposed to two types of sequential treatments, at 21 °C: 1) 30s of 222 nm UV+48h of 405 nm blue light; 2) 30s of 280 nm+48h of 405 nm blue light. Single wavelength treatments were also conducted under the same conditions. Confocal microscopy was used to visualize bacteria distribution and quantify oxidative cellular damage. Experiments were performed in triplicate with independently grown cultures, and data was statistically analyzed.

Results: Inactivation curves for all treatments were non-linear, and were described by the Weibull model ($0.97>R^{2>0.82}$). Kinetics were significantly ($p<0.05$) affected by bacteria species. Synergistic effects were observed for *E. coli* and *Listeria* after exposure to both sequential treatments, with > 3 log reduction increase compared to single wavelength treatments. Additive effects were observed for *S. aureus*, while antagonistic effects were observed for *P. aeruginosa* after sequential treatments. Significant differences in reactive oxygen accumulation were found ($p<0.05$) among treatments. Mechanisms and performances of sequential light treatments were correlated with cellular oxidative damage.

Significance: The proposed sequential treatments showed enhanced disinfection performance compared to individual treatments in most cases, which can have significant food safety benefits.

P1-80 Evaluation of *in Vitro* Biofilm Formation of *Salmonella enterica* from Different Sources

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella enterica* can form biofilms for its survival. This response could be promoted depending on the isolation source.

Purpose: To evaluate the *in vitro* biofilm formation (BF) of *S. enterica* strains isolated from different origins in Mexico.

Methods: The *in vitro* BF of 90 *S. enterica* strains isolated from different sources (plant-based foods, food not specified, human, environment) was evaluated by crystal violet staining assay in microplates at 25°C through the time. A 96-well microplate was used, and 180 µL of tryptic soy broth was added to each well and inoculated with 20 µL of *S. enterica* strains (~$1x10^5$ CFU/mL). The BF was evaluated at 24, 48, and 72 h. Finally, the BF was classified into four groups: none, weak, moderate, and strong producers. Differences among the origin of strains, quantity of biopolymers, and BF time was evaluated by ANOVA and Tukey test.

Results: High variability in the BF among *S. enterica* strains was observed through the time (p<0.05), varied from 0.11 to 2.19 OD595nm at 24h, from 0.09 to 3.04 OD595nm at 48h, and from 0.07 to 4.05 OD595nm at 72h. Generally, high BF was observed at 72 h and in human isolates (p<0.05). The *S. enterica* isolates at different times were classified mainly as non-producers (41.1 to 73.3%).

Significance: The *S. enterica* strains isolated from humans had the highest BF through time, suggesting that this could be an essential characteristic to consider to evaluate their virulence.

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P1-81 Exposure of Monophasic *Salmonella* Typhimurium to Benzalkonium Chloride Leads to Acquired Resistance to This Disinfectant and Antibiotics

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Introduction: *Salmonella* resistance to antimicrobial agents is an important global public health problem. Extensive use of disinfectants such as benzalkonium chloride (BC) has imposed a selective pressure and may contribute to the emergence of disinfectant- and antibiotic-resistant *Salmonella*.
Purpose: This work aimed to investigate whether continuous exposure of monophasic *Salmonella* Typhimurium (*S*. 1,4,[5],12:i:-) to BC could lead to adaptive resistance and cross-resistance, and to explore potential resistance mechanisms.
Methods: Twelve clinical and food *S*. 1,4,[5],12:i:- isolates susceptible to BC were selected and were continuously cultured in a Luria-Bertani medium with gradually increasing concentrations of BC. The stability of adaptive resistance was determined in each BC-adapted strain after five passages in a BC-free medium. The minimum inhibitory concentrations (MICs) for BC and antibiotics against adapted strains were assayed by the broth microdilution method. Mechanisms of acquired resistance to BC were analyzed by characterizing cell surface properties. One-way analysis of variance and Duncan's test ($P<0.05$) were used for statistical analysis.
Results: BC tolerance increased in all tested strains, with increases in MICs between two and six-fold. Adaptive resistance was stable after five passages in a BC-free medium. The survival rate of BC-adapted strains was significantly ($P<0.05$) higher than their wild-type counterparts in lethal concentrations of BC. Most BC-adapted strains showed increased resistance to tigecycline, ciprofloxacin, levofloxacin, and cefazolin. Significant reductions ($P<0.05$) in zeta potential and cell surface hydrophobicity were observed in BC-adapted strains compared to their wild-type ones, indicating that changes in cell surface properties were a cause of adaptive resistance.
Significance: These results demonstrate that repeated exposure to BC could result in the emergence of BC- and antibiotic-resistant *S*. 1,4,[5],12:i:- strains, which may represent an increased risk for public health.

P1-82 Microbial Inspection of Edible Insect Products Available for Human Consumption within the United States

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❖ ***Developing Scientist Entrant***

Introduction: Edible insects are promising source of protein for humans. However, the food safety risks of edible insects were not examined before within the US.
Purpose: We aimed to investigate the microbial content of ready-to-eat edible insect products.
Methods: For this study, we purchased a total of 8 kinds of edible insect products (Diving Beetles, Silkworm, Grasshoppers, Jamaican Crickets, Mealworms, Mole Crickets, House Crickets, and House Cricket Powder). Three product samples for each product type were used for microbial counts analysis. Using traditional microbiological methods, enumerations for total viable (cell) counts **(TVC)**, bacterial endospores **(BS)**, *Lactobacillaceae* **(LB)**, and *Enterobacteriaceae* **(EB)** were obtained for each product type in triplicate. Whole genome sequencing **(WGS)** was used to further characterize selected colonies (n = 96) of the enumeration method. Microbial counts data were analyzed using one-way ANOVA ($\alpha = 0.05$) in R and sequence data were taxonomically classified to organism using Sepia. *Bacillus cereus* group isolates were further characterized using Btyper3.
Results: TVC, BS, and LB levels differed by product type ($P = 0.017$, $P = 0.029$, and $P = 0.054$, respectively). Numerically, the highest counts of TVC, BS, and LB were found in Mole Crickets (6.01 log CFU/g), Mole Crickets (5.25 log CFU/g), and House Cricket Powder (4.86 log CFU/g), respectively. EB were only observed in Mole Crickets (2.30 log CFU/g) and House Cricket Powder (2.15 log CFU/g). WGS revealed most sequenced isolates belong to the *Bacillus cereus* and *B. subtilis* groups. Btyper3 analysis of the *B. cereus* group isolates identified some isolates from Mole Crickets as biovar Emeticus.
Significance: While edible insects may provide an excellent food alternative for protein, the presence of *B. cereus* group bacteria, in particular emetic strains, may pose food safety concerns.

P1-83 Isolation and Genomic Characterization of a *Cronobacter sakazakii* Sequence Type 64 Strain from Chili Powder

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Introduction: *Cronobacter sakazakii* is an emerging, opportunistic, Gram-negative, foodborne bacterium that can cause life-threatening meningitis and necrotizing enterocolitis, predominantly in neonatal, 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 range of foods. So far, application of whole genome sequencing (WGS) has advanced bacterial typing and has been extensively utilized for precise strain identification to understand the transmission dynamics of disease. In this study, we describe the draft genome sequence of a *Cronobacter sakazakii* strain SRL-109, recovered from chili powder.
Purpose: The major objective of this study was to identify the human pathogenic *Cronobacter sakazakii* strain isolated from spice samples by performing WGS analysis.
Methods: In this study, chili powder from South America was analyzed, and a *Cronobacter sakazakii*-like bacterial strain was isolated. Preliminary identification of this isolate was achieved by employing the VITEK 2 system, real-time PCR assay and MALDI-TOF MS analysis, following FDA's Bacteriological Analytical Manual and manufacturer's protocols. 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 procedures.
Results: Species identification with high confidence value (>99%) was observed for the *Cronobacter sakazakii* strain SRL-109 while performing MALD-TOF MS analysis. Genome sequence of this strain was 4,495,336 bp in length, and the draft genome was distributed in 44 contigs. WGS data analysis revealed the sequence type as ST64 for the genome of *Cronobacter sakazakii* strain SRL-109.
Significance: *Cronobacter sakazakii* ST64 strain has also been isolated from raw material, the environment, and manufacturing facilities of PIF and other foods. WGS methodologies can be applied for correct strain identification of foodborne *Cronobacter sakazakii* isolates known to contaminate foods including PIF, causing serious illness in humans.

P1-84 Postbiotics: Considerations for Safety and Quality Management

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Introduction: Postbiotics are preparations of dead microorganisms and/or their components that confer a health benefit on the consumer. They are a rapidly growing (11.5 % compound annual growth rate, 2022-32) emerging category of functional ingredients in part because of their safety profiles, but pose challenges related to defining efficacy and measuring potency compared to their traditional probiotic counterparts.
Purpose: To demonstrate a proof of concept for a reliable method to evaluate postbiotic concentrations by flow cytometry.
Methods: Commercial postbiotic materials were evaluated by flow cytometry following ISO 19344 Protocol B, a viability assay based on cell membrane integrity using dual nucleic acid stains SYTO24 and Propidium Iodide (PI). PI cannot penetrate intact cell membranes, but penetrates damaged membranes and binds to the cellular DNA, indicating cell death or injury. The test separates the populations (live, injured, dead) through the different emission spectra

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of bound and unbound PI. Direct microscopic counting using a hemocytometer was used to determine baseline concentration of dead microorganisms present in the samples. Five test samples at different concentrations were each tested five times by both flow cytometry and direct cell counting.

Results: The average concentration of dead cells across the five replicates of each of the five samples was approximately 7.0×10^7, 7.0×10^8, 7.0×10^9, 7.0×10^{10} and 7.0×10^{11} per g by both flow cytometry and direct microscopic count, spanning a range of four logs. Linear regression analysis of the log-transformed results found a correlation coefficient >95%, which indicates that flow cytometry can accurately determine postbiotic concentration in a pure material.

Significance: We believe that this is the first demonstration of flow cytometry as a powerful tool to measure concentration of postbiotic materials, a critical step in ensuring product quality and delivering to consumers the benefits they expect. This is a significant advance over laborious direct microscopic counting and useless viable counting methods.

P1-85 Synergistic Effects of ε-Poly-L-Lysine and Lysozyme Against *Pseudomonas aeruginosa* and *Listeria monocytogenes* Biofilms on Beef and Food Contact Surfaces

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Introduction: *Listeria monocytogenes* is one the most common pathogens encountered, while *Pseudomonas aeruginosa* is an opportunistic pathogen. The natural antibacterial peptide and enzyme-based techniques have attracted much attention in the food, biomedical, and chemical industries due to wide antibacterial spectrum and safety.

Purpose: The purpose of this study was to evaluate the antibiofilm activity of ε-poly-L-lysine (ε-PL) and lysozyme and investigate the effective combination treatment against *P. aeruginosa* and *L. monocytogenes* biofilms on beef and food contact surfaces.

Methods: The antibiofilm activity was conducted by the minimum inhibitory concentration and antibiofilm assessment. The visual examinations were performed by field emission scanning electron microscopy. Quality test of color and texture was also conducted on beef. The inhibition ability of ε-PL and lysozyme was analyzed by genomic expression analysis by quantitative real-time polymerase chain reaction and fourier-transform infrared spectroscopy (FTIR) analysis of the extracellular polymeric substance.

Results: The present study revealed that maximum biofilm reduction was 3.1 log CFU/cm^2 from stainless steel after individual treatment with ε-PL against *L. monocytogenes*. However, sequential treatment of ε-PL and lysozyme led to biofilm reductions of 5.6, 5.7 log CFU/cm^2 and 4.2 log CFU/g on silicon rubber, stainless steel, and beef, respectively. Visual examinations supported the reduction of biofilms. No difference was showed the quality of the food surfaces after the treatment of ε-PL and lysozyme. Both ε-PL and lysozyme suppressed the relative expression levels of quorum sensing and virulence regulatory genes. In addition, FTIR analysis confirmed that ε-PL and lysozyme caused biofilm matrix destruction.

Significance: This research suggested that combined use of ε-PL and lysozyme effectively reduced *P. aeruginosa* and *L. monocytogenes* biofilm associated with cross-contamination in raw beef processing.

P1-86 Evaluation of a Rapid qPCR Automated Method with Reduced Enrichment Time for Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) in a Brazilian Beef Producer Industrial Laboratory

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Introduction: Time required for food pathogen detection is critical in industrial laboratories analysis routine. Therefore, fast methods with high sensitivity and minimum analyst intervention are of great interest for the food industry.

Purpose: Evaluation of an automated qPCR method with reduced enrichment time for Top Seven STEC detection in meat and environmental samples within the industrial laboratory routine.

Methods: 375 g of raw beef trim (n=40), 375 g of hamburger (n=40) and surface swab (n=40) were divided into four groups and inoculated with individual STEC serogroups at low levels (≤7 CFU/sample for O157:H7 (depending on the sample) and ~10 CFU/sample for other serogroups), as follows: Group 1: O157:H7 and O111; Group 2: O26, O103 and O145; Group 3: O45 and O121; Group 4: Negative control, without STEC. Additionally, all samples were inoculated with *Klebsiella aerogenes* (1000 CFU/sample) as a competitor. Samples were diluted 10-fold in pre-warmed BPW and enriched at 41°C for 8 h. Then, the extraction and preparation of the qPCR plates were performed by the iQ-Check Prep Automation System using qPCR STEC kits for screening (presence/absence of *stx* and *eae* genes) and serogroups confirmation.

Results: The STEC screening method effectiveness was observed in less than 12 h, including incubation time, and serogroups confirmation after additional 2 h of analysis. The method was able to detect O157:H7 inoculated at ~7 CFU/sample in 100% of hamburger samples and in 50% of meat samples and surface swabs inoculated at 0.6 CFU/sample. The latter was observed due to the low concentration of this serogroup in samples.

Significance: These results demonstrated the high specificity of the automated qPCR method with shortened STEC enrichment protocols in a routine industrial laboratory, contributing to reduced time to results, sample handling and cross contamination probability.

P1-87 Resolving Contamination by Shiga Toxin-Producing *Escherichia coli* from Mixed Cultures of Interfering *E. coli* Possessing Either Shiga Toxin or Intimin Genes

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Introduction: The USDA-FSIS tests raw beef products for adulterant Shiga toxin-producing *E. coli* (STEC) belonging to the "top-seven" O-antigen serogroups and contain Shiga toxin (*stx)* and intimin (*eae)* genes. Detecting STEC becomes challenging in the presence of mixtures of interfering *E. coli* that possess only one of the target genes because the tests cannot tell if the genes originate from separate bacteria. However, digital droplet PCR (ddPCR) is performed on whole *E. coli* and can determine if the genes are linked within the same cell.

Purpose: Apply a commercial ddPCR to resolve mixed cultures from adulterant or *stx*$^+$/*eae*$^+$ STEC in beef.

Methods: Beef trimmings were inoculated with combinations of *stx*$^+$, and *eae*$^+$, or *stx*$^+$/*eae*$^+$ *E. coli* of various serogroups and enriched (42°C, 16h) in buffered peptone water (BPW; n=36) or modified tryptic soy broth (mTBS; n=36). Beef enrichment broths that were identified as *stx* and *eae* positive (n=95) were provided by FSIS. Broths were screened for *stx* and *eae* using Bio-Rad iQ-Check STEC test kits, with positives tested using ddPCR (Bio-Rad dd-Check STEC) kits. All broths were cultured for STEC using a combination of immunomagnetic concentration and direct plating.

Results: Fifty-four of the 72 inoculated broths and 83 of the 95 regulatory broths screened *stx* and *eae* positive by Bio-Rad iQ-Check STEC tests. ddPCR resolved correctly all but one (98%) inoculated broth as either containing a STEC or a mixed culture. Amongst the 89 regulatory broths 11 were culture confirmed. ddPCR identified 8 as positive or potential positive, 2 negative, and 1 invalid. The positives included STEC of serogroups O103, O5, and O182. ddPCR further identified 66 non-cultured confirmed broths as mixed cultures.

Significance: ddPCR helps distinguish mixed cultures from broths containing STEC. This increased accuracy allows more efficient use of labor and resources to isolate and identify STEC.

P1-88 Optimization of a Propidium Monoazide-Quantitative PCR Method for Quantification of Viable-but-Non-Culturable *Campylobacter jejuni* in Poultry Products

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Introduction: Many pathogenic bacteria including *Campylobacter* can enter the viable-but-non-culturable (VBNC) state under unfavorable conditions. VBNC state of pathogens can pose risks to food safety and public health because they cannot be detected by conventional microbiological culture-based methods but resuscitate under favorable conditions to develop virulence.

Purpose: This study aimed to develop an optimized propidium monoazide (PMA)-quantitative PCR method for the rapid and accurate determination of VBNC *C. jejuni* in agri-food products.

Methods: Live (6 log CFU/mL) and dead (6 log CFU/mL, heat inactivated at 90°C for 5 min) *C. jejuni* cells were separately treated by different concentrations of PMA (0, 10, 15, 20, 50, and 100 μM) and the optimal concentration of PMA (20 μM) was used for qPCR assay. Then, the sensitivity of PMA-qPCR assay was evaluated by detecting viable *C. jejuni* in live-dead bacterial mixtures containing live cells ranging from 2 to 8 log CFU/mL and dead cells of 6 log CFU/mL. In addition, the induction process of VBNC *C. jejuni* under osmotic stress (7% NaCl) was monitored by PMA-qPCR and the plating assay. Moreover, the applicability of this method was evaluated by detecting VBNC *C. jejuni* spiked in chicken breast samples.

Results: A standard curve with a background of heat-inactivated *C. jejuni* cells was obtained with a linear quantification range of 3.43 to 8.43 log CFU/mL and a correlation coefficient of 0.9999. In addition, over 10% *C. jejuni* population was successfully induced into the VBNC state after 48-h treatment. As for the application in poultry products, the limit of detection was determined to be 3.12 log CFU/g.

Significance: We developed a rapid, specific, and sensitive method for the detection and quantification of VBNC *C. jejuni* in poultry products using PMA-qPCR, providing a powerful tool to assess the prevalence of VBNC *C. jejuni* in the agro-ecosystem.

P1-89 Development of Real-Time Polymerase Chain Reaction Method for Rapid Detection and Quantification of Probiotics Based on Pan-Genome Analysis

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Introduction: Several recent clinical trials have reported a potential risk of probiotics. In addition, some commercially available probiotics products may cause contamination accidents in which the displayed strain and the actual strain are different.

Purpose: To solve this problem, the unique gene of each species was designated based on pan-genome analysis to accurately detect the probiotics species. And a real-time PCR method was developed to rapidly identify and quantify each probiotic species.

Methods: To select the target gene of each bacteria, pan-genome analysis was performed with a total of 4,345 complete genome sequences. Selected genes were validated across other bacterial genus using BLASTN. After designing primer and probe sets with species-specific genes, these sets were validated by single plex, crosscheck, and multiplex PCR methods in each conventional PCR and Real-time PCR method (triplicates). Then we constructed the standard curve in real-time PCR to quantify each species. To verify the accuracy of the standard curve through experiments for food application, a model was created in which 1×10^8 CFU/ml strains were inoculated in sterile milk and the real-time PCR and direct counting method were compared.

Results: According to conventional PCR and real-time PCR, primer and probe sets successfully distinguished each species. Standard curves test results, slopes for the specific primers ranged from -3.00 to -3.50 and the lowest R^2 value of the standard curve was >0.991. With these standard curves, we performed real-time PCR and viable cell count and compared them using Mann-Whitney U test. There were no significant differences between real-time PCR and the viable cell count method.

Significance: Existing culture-based probiotics identification methods are difficult to measure the number of CFUs by type of mixed strain products, but the newly developed method can measure them possibly. These advances are thought to have implications for increasing the safety of probiotic products.

P1-90 Matrix Validation of Almond Milk for *E. coli* O157:H7 and *Salmonella* Using the Hygiena® BAX® System

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Introduction: There are an abundance of plant-based alternative milks in the market today produced from various nuts and seeds. Raw ingredients used to manufacture these products, such as almonds, have the potential to be contaminated with foodborne pathogens.

Purpose: The objective of this study was to evaluate the performance of a rapid PCR method for the detection of *E. coli* O157:H7 and *Salmonella* from a single enrichment of 375 mL of almond milk.

Methods: An unpaired matrix validation for almond milk was performed following the technical guidelines in Appendix J of the AOAC INTERNATIONAL Official Methods of Analysis to compare two commercial real-time PCR assays to the ISO reference methods for the detection of *E. coli* O157:H7 and *Salmonella*. Test portions were co-inoculated with *E. coli* O157:H7 and *Salmonella* at a low level (0.2 to 2 CFU/test portion) and a high level (≥ 5 CFU/test portion) and then stored at 4 °C for 48 to 72 hours. Test method samples (375 mL) were enriched in BPW and incubated for 12-24 hours before being tested by real-time PCR and culture confirmed. Reference method samples (25 mL) for each organism were enriched and confirmed according to their respective ISO procedures.

Results: Test method samples analyzed by real-time PCR detected 13/20 positives for *E. coli* O157:H7 and 10/20 positives for *Salmonella* in the low inoculum level samples. All 5 high-level spiked samples were positive. These results were in complete agreement with culture with 100% sensitivity and 100% specificity. When compared to the reference methods, the difference in probability of detection (dPOD) indicated no significant difference for either organism.

Significance: This study shows that the BAX® System is specific, sensitive, and accurate for the detection of *E. coli* O157:H7 and *Salmonella* in 375 mL samples of almond milk using a single enrichment.

P1-91 Validation of Five Powdered Spices for the Detection of *Listeria* Using the Hygiena® BAX® System

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Introduction: The recovery and detection of pathogens in spices remain a challenge due to various active, naturally occurring, antimicrobial compounds. Gram-positive organisms are more susceptible compared to Gram-negative organisms due to the lack of an outer membrane allowing these phytochemicals direct interaction with the cell membrane.

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 five powdered spices.

Methods: Garlic, onion, parsley, red pepper and paprika were each tested in separate studies. Spices were weighed into 25 g test portions and enriched in either TSB with K_2SO_3 for garlic and onion or 24 LEB Complete for parsley, red pepper and paprika. After the media was added, samples were inoculated with *Listeria monocytogenes* to create 20 low-level samples and five high-level samples. Following inoculation, samples were incubated at 35 °C. A second-

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ary transfer into MOPS-BLEB was included after 24 hours for garlic only. Samples were analyzed by real-time PCR and confirmed according to procedures in the FDA BAM Chapter 10.

Results: At the low inoculation level, real-time PCR detected *Listeria* in 8/20 for garlic, 13/20 for onion and parsley, 18/20 for red pepper and 6/20 for paprika samples. All high-level samples were positive for each matrix. All presumptive positive results were identical to culture with 100% sensitivity and 100% specificity.

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 25 g samples of garlic powder, onion powder, parsley powder, red pepper powder and paprika, statistically equivalent to the reference culture method.

P1-92 *Salmonella* Species PCR Assay Method ISO 16140-2:2016 Matrix Extensions

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Introduction: *Salmonella* is a major global foodborne pathogen with a severe impact on 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 and environmental surfaces (validated in accordance with ISO 16140-2:2016). The scope and capability of the method has been extended by adding pet food and animal feed matrixes and enhancing protocols for meat, vegetable and powdered infant formula (PIF) categories.

Purpose: Perform ISO 16140-2:2016 extension studies to include pet food and animal feed matrixes, and enhance the protocol for meats, vegetables and PIF by increasing the sample size and reducing the time to result.

Methods: In accordance with ISO 16140-2:2016 a sensitivity study and relative level of detection (RLOD) were conducted for each category (meat 25g and 375g, vegetables 375g, pet food 375g, animal feed 150g, and powdered infant formula (PIF) 375g). Pet food was tested with both a paired and unpaired design, all other categories used an unpaired design.

Results: The results of each category for the sensitivity and RLOD studies met the ISO 16140-2:2016 standard requirements meaning that the performance of the SureTect™ Salmonella species method was statistically equivalent or better than the performance of the reference method.

Significance: The SureTect™ Salmonella species PCR Assay was proven to be an accurate and reliable method for the detection of *Salmonella* from the additional matrices, and the new protocol for meat, vegetable and PIF categories.

P1-93 An ISO 16140-2:2016 Extension Study for a *Cronobacter* Species PCR Assay to Include 375 g Powdered Infant Formula, Infant Cereals and Related Ingredient Matrices

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Introduction: *Cronobacter* spp. are opportunistic pathogens predominantly found in dried powders, specifically powdered infant formula (PIF). *Cronobacter* infections are of concern for patients with weakened immune systems particularly neonates, with case mortality reported to be between 50-80% for those at-risk groups. Therefore, detection of *Cronobacter* spp. before contaminated foodstuffs reach patients is of marked importance.

Purpose: To extend the scope of validated matrices for the Thermo Scientific™ SureTect™ Cronobacter PCR Assay (alternative method) to include up to 375 g PIF with and without probiotics and infant cereals and related ingredients.

Methods: The alternative method was examined against the ISO 22964:2017 reference method using an unpaired study design. The sensitivity study comprised of 66 samples for the extension study PIF category, and the relative limit of detection (RLOD) study comprised of 30 samples across three levels of contamination. Inclusivity/exclusivity data from the initial validation study was used, which comprised of 57 inclusivity isolates and 31 non-target strains.

Results: The sensitivity study detected 7 positive deviations compared to 4 negative deviations, meaning the sensitivity study was below the acceptability limit (AL) of 3 for an unpaired study. The RLOD study data was below the AL of 2.5 for unpaired studies showing that the alternative and reference method perform comparably. The inclusivity/exclusivity study successfully detected and excluded all target and non-target isolates during the initial validation.

Significance: The alternative method demonstrates comparable performance to the reference method and was granted NF VALIDATION status. The alternative method constitutes a rapid and reliable workflow for the detection of *Cronobacter* spp. from up to 375 g infant formula with and without probiotics, and also allows for enrichment harmonisation for *Salmonella* testing.

P1-94 AOAC PTM Extension Study to Validate the Surecount *Salmonella* Multiplex PCR Kit for the Quantification of *Salmonella* Species, *Salmonella* Typhimurium, and *Salmonella* Enteritidis

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Introduction: *Salmonella* causes an estimated 1.35 million infections annually in the United States according to Centers for Disease Control data. Different action plans across the food supply chain, including quantitative risk assessment and surveillance of serovars most frequently associated with human disease, are in place to reduce prevalence of *Salmonella.* Very low levels of *Salmonella* are typically found in food and environmental samples; cost-effective and easy-to-handle methods capable of enumerating low levels of *Salmonella* are needed to identify critical contamination points together with inactivation process efficiency.

Purpose: To evaluate the Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis PCR kit for rapid multiplex quantification of *Salmonella* from selected meat matrices according to the AOAC *Performance Tested Methods*℠ program

Methods: A matrix study consisting of 375 g ground turkey, 375 g ground beef, 375 g ground pork and chicken carcass rinse was conducted according to AOAC Appendix J against the USDA FSIS MLG 4.11 MPN method. Three matrices (turkey, chicken carcass and pork) were challenged with serotype co-infection to demonstrate the sensitivity and specificity of the multiplex assay. Inclusivity/exclusivity studies were carried out which consisted of at least 50 target *Salmonella* per serotype (200 total) and 15 exclusivity isolates.

Results: The candidate method demonstrated either equivalent ($\log_{10}$ CFU/mL within 0.5 log of the reference method and within the stipulated -0.5, 0.5 90% confidence interval) or comparable performance to the reference method. Grubbs tests showed outliers at different spike levels for all matrices, but this did not impact results. Inclusivity of the assay was 99.5%.

Significance: The performance of the candidate method met all Appendix J requirements and constitutes a rapid and reliable alternative method for the quantification of *Salmonella* spp. and specific serovars Typhimurium and Enteritidis within 8 hours.

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P1-95 Method Modification Validation of the *Listeria* Detection and Enumeration Methods in Accordance with ISO 16140-2:2016

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Introduction: *Listeria monocytogenes* is a major global foodborne pathogen with a severe impact on public health. The ISO 16140-2:2016 validated Thermo Scientific™ Oxoid™ Listeria Precis™ detection and enumeration methods have been extended to offer simpler workflows, improved time to result, and greater flexibility for the detection and enumeration of *Listeria* species and *L. monocytogenes*.

Purpose: Perform ISO 16140-2:2016 studies to validate the workflow improvements of the Listeria Precis™ methods.

Methods: The method modifications were validated against the 2017 ISO 11290 part 1 and part 2 reference methods in an unpaired study design. For detection, the method consisted of a 20-hour enrichment using a proprietary enrichment broth followed by streaking 10µL on Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (ISO). For enumeration, the method consisted of a dilution step followed by plating on *Brilliance*™ Listeria Agar (ISO). All presumptive colonies on *Brilliance*™ Listeria Agar (ISO) were confirmed using the appropriate tests, including the rapid Thermo Scientific™ PrecisCheck™ lateral flow test.

Results: For detection, the sensitivity of the method for the detection of *L. monocytogenes* was 90.4% compared to 89.3% for the reference method. For *Listeria* species, the sensitivity was 91.2% and for the reference method it was 87.4%. For enumeration, the average difference in the relative trueness studies for *L. monocytogenes* were -0.02 log cfu/g with the pour plate protocol and 0.02 log cfu/g with the surface plate protocol. For *Listeria* species, the average difference was -0.03 log cfu/g with the pour plate protocol and 0.00 log cfu/g with the surface plate protocol.

Significance: The detection and enumeration Listeria Precis™ methods for *Listeria* species and *L. monocytogenes* provided satisfactory results in the ISO 16140-2:2016 validation studies, demonstrating that they are reliable and efficient methods for the detection and enumeration of *Listeria* from foods and environmental surfaces.

P1-96 Validation of a Rapid Culture Media Workflow According to ISO 16140-2:2016 for the Detection of *Cronobacter* spp. from Selected Matrices

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Introduction: *Cronobacter* species are ubiquitous organisms that are found in dried powders, specifically powdered infant formula (PIF). *Cronobacter* infections are particularly concerning for patients with lower immune systems such as neonates, with case mortality reported to be between 50-80% for those at-risk groups.

Purpose: To evaluate the Thermo Scientific™ Cronobacter Precis™ method (alternative method) according to the EN ISO 16140-2:2016 for detection of *Cronobacter* in PIF and environmental samples for MicroVal accreditation.

Methods: The validation study evaluated 10 g PIF (with and without probiotics) against the ISO 22964:2017 reference method using a paired study design, and 375 g PIF (with and without probiotics) and 25 g environmental samples using an unpaired study design. The sensitivity study contained a total of 199 samples according to ISO 16140-2 guidelines. The relative limit of detection (RLOD) study contained 3 categories, with 3 levels of contamination per category. The inclusivity and exclusivity analysed 57 and 31 isolates, respectively.

Results: The sensitivity study gave a total of 12 positive deviations and 7 negative deviations which were below the acceptability limits (AL) for each category, showing comparable performance to the reference method. The alternative method demonstrates improved sensitivity (92.3%) compared to the reference method (86.8%). For the RLOD study, the values were below the AL of 2.5 for an unpaired study and 1.5 for a paired study demonstrating comparable detection to the reference method. All study data met the ISO 16140-2:2016 requirements, and the alternative method was granted MicroVal certification.

Significance: The alternative method constitutes a rapid and reliable workflow for the detection of *Cronobacter* spp. from PIF with and without probiotics and environmental samples and provides a markedly improved time to result compared to traditional reference techniques.

P1-97 Comparative Evaluation of Loop-Mediated Isothermal Amplification (LAMP) Bioluminescent Assay and ISO 11290-1 for Detection of *Listeria monocytogenes* in Powdered Infant Formula

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Introduction: Infant formulas represent one of the most highly regulated foods because they serve as sole source of nutrition for infants at a critical stage of their growth and development. Contamination issues with pathogens such as *Listeria monocytogenes* can pose a significant risk to this highly vulnerable group. Rapid and accurate pathogen detection in this food matrix is essential to verify effectiveness of control measures during production and ensure the safety of finished products.

Purpose: This study evaluated loop-mediated isothermal amplification (LAMP) assay for the detection of *Listeria monocytogenes* in 125-g powdered infant formula and compare its performance against reference method ISO 11290-1.

Methods: The method comparison study was performed according to ISO 16140-2, using powdered infant formula as test matrix. Different sample sizes (25-g and 125-g) of three different types of powdered infant formula (goat, cow and plant-based) were spiked with *Listeria monocytogenes* ATCC 49594 ranging from <0.1 CFU and 20 CFU per test portion. Samples were enriched in Demi-Fraser Broth at 1:10 dilution and incubated at 30°C for 24-30 h, followed by secondary enrichment in Fraser Broth. Analyses for *L. monocytogenes* was performed using both LAMP assay and cultural confirmation.

Results: The LOD50 for *L. monocytogenes* in 125-g infant formula was 2.10 using LAMP method and 1.55 CFU using cultural procedure, respectively. The LOD50 for 25-g sample using reference ISO 11290-1 procedure was 0.95 CFU. Relative limit of detection (RLOD50) value when comparing the alternative pooled size (125 g) against the reference sample size (25 g) was 2.2 (<2.5), indicating equivalency between the two methods.

Significance: The results showed that LAMP-based bioluminescent assay can be an effective method for rapid detection of *L. monocytogenes* in powdered infant formula. Successful detection of this pathogen in pooled test portions of infant formula up to 125g can be achieved, provided the maximum incubation time was followed.

P1-98 ISO 16140-2 (2016) Method Comparison of Iq-Check STEC Virx Method for the Detection of Shiga-Toxin-Producing *Escherichia coli* (STEC) in Flours and Raw Dough Products

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Introduction: Recently, flour products were described as a source of contamination for Shiga-toxin-producing *Escherichia coli* (STEC) with several outbreaks occurring in the US and in Europe. While alternative methods exist for STEC detection, none was validated according to ISO 16140-2 for this food category.

Purpose: For the first time, an independent extension study compared an alternative method for the detection of STEC in flour products to the ISO/TS 13136:2012 (reference method) according to the ISO 16140-2:2016.

Methods: The alternative method is a real-time PCR assay (iQ-Check STEC VirX, Bio-Rad) targeting *stx* virulence genes. After an enrichment of 375g test portions diluted (1:4) in supplemented Buffered Peptone Water, incubation at 41.5±1°C during 18h prior to DNA extraction and PCR screening was

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performed. The alternative method was compared to ISO/TS 13136:2012 to evaluate its sensitivity and relative level of detection (RLOD). A large variety of flour and raw dough products and STEC strains were evaluated.

Results: Overall, 63 samples were analysed for the sensitivity study, providing 34 *stx* positives on the alternative method and 27 positives with the reference method. Among those positives 7 samples were naturally contaminated. The sensitivity was estimated at 84.6% and 69.2% for the alternative and reference methods respectively. The RLOD study performed on pizza raw dough indicated equivalent level of detection for both methods (RLOD= 1.170).

Significance: Equivalent or improved performances were observed for the alternative method leading to its validation by MicroVal Technical Committee. The availability of a validated alternative method is key to facilitate the method implementation and STEC surveillance of this food category by routine laboratories.

P1-99 Towards the Detection of the Most Dangerous Strains of Shiga-Toxigenic *Escherichia coli* in Mixed Culture

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Introduction: In 2018, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) reported that concomitant presence of specific alleles of Shiga toxin *stx* (*1a, 2a, 2c, 2d*) with the adhesin *eae* greatly increased the risk of STEC strains to cause potentially fatal hemolytic uremic syndrome (HUS). Risk assessment for foodborne STEC is currently done after WGS analysis of isolates, approximately 6 to 10 days after a product is first tested. Newly developed advanced multiplexing capabilities using digital PCR (dPCR) presents the possibility to surveil for multiple gene targets within the same cell in mixed cultures.

Purpose: Primers specific to the STEC virulence genes listed above were designed and validated as a first step towards designing a targeted multiplex dPCR assay that can detect the most dangerous STEC strains in enrichment samples.

Methods: Oligonucleotide primers were designed by Clone Manager 9 software. After individual testing, the best performing primers were combined in a 50µl PCR-assay and analyzed on E-gel agarose gels after 35 cycles of amplification. One-hundred-twenty-eight genetically diverse STEC strains with known virulence genotypes, plus 64 *E. coli* strains including 22 capturing *eae* diversity, comprised the inclusivity strain panel. Fifty-five non-STEC strains were used in the exclusivity strain panel.

Results: We successfully developed a multiplex assay that amplified *stx2a*, *stx2c*, *stx2d (*586bp), *stx1a (305bp*), and *eae* (152bp), in mixed cultures in the presence of DNA lysates prepared from spinach and lettuce enrichments. *E. coli uidA* (206bp) served as an amplification positive control. Except for 2 *stx2c* strains, no false positive or false negative products were amplified.

Significance: Early detection of potential HUS causing STEC benefits public health and provides valuable information for perishable-sensitive food products and in situations where STEC are not recovered from PCR-positive samples. With the addition of probes, the primers developed here will be adapted for a dPCR multiplex assay.

P1-100 Different Tolerance of Loop-Mediated Isothermal Amplification and Polymerase Chain Reaction to Inhibitors in Chicken Carcass Rinse and Feces for Detecting *Campylobacter jejuni*

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Introduction: *Campylobacter* species, particularly *C. jejuni*, are a leading cause of human foodborne gastroenteritis worldwide, with poultry being the major reservoir. Rapid molecular methods are needed to detect this organism in poultry for baseline studies and the reduction of this pathogen in poultry and poultry products. Polymerase chain reaction (PCR) is a commonly used to detect foodborne campylobacters. However, inhibition of PCR due to inhibitors in various types of samples has been problematic. A novel loop-mediated isothermal amplification (LAMP) procedure using a different polymerase has been shown to be less affected by the inhibitory substances compared with PCR.

Purpose: This study investigated the relative tolerance of LAMP and real-time PCR detecting *C. jejuni* to inhibitors in chicken carcass rinse and feces for rapid detection with or without DNA extraction steps.

Methods: Chicken carcass rinses and feces were experimentally spiked with *C. jejuni* (10^1 – 10^9 colony forming unit CFU/ml). Detection sensitivities of PCR and LAMP using purified DNA or lysed samples were compared in at least two experiments.

Results: The PCR and LAMP procedures showed a similar detection sensitivities using purified DNA (approximately 10^1-10^2 CFU/ml) extracted from contaminated chicken carcass rinses and fecal homogenates and using lysed samples (10^4 and 10^5 CFU/ml respectively) from contaminated chicken carcass rinses. When testing lysed samples from contaminated fecal homogenates (n=10), LAMP demonstrated a detection limit at 10^4 CFU/ml, however, PCR showed negative results for all concentrations.

Significance: This study demonstrates that LAMP is more tolerant to the inhibitors in chicken fecal samples than that of PCR. The LAMP procedure could be complementary to PCR when different types of inhibitors are present, and could also be an alternative, simple and less expensive test amenable for use under various testing conditions.

P1-101 Bio-Mapping of *Salmonella* Levels Comparison between Two PCR Methods of Quantification and Detection in a Commercial Poultry Processing Facility in the United States

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Introduction: Pathogen control in poultry processing facilities, requires sound data on the pathogen loads at different stages of processing for risk-based decision-making, and to assist processors in compliance with regulatory performance standards using accurate methods.

Purpose: To develop a product comparison for levels of *Salmonella* doing a baseline in a commercial poultry processing facility operating under the NPIS.

Methods: A poultry processing facility, was sampled during 3-week period following the sampling scheme of the USDA-FSIS performance standards. Six locations were sampled: Rehang, Post Pick, Pre chill , Post chill and parts: Wings pre intervention and Wings post intervention. At each location, 12 samples were taken per repetition. A total of 36 samples were taken. *Salmonella* counts were determined using BAX® SalQuant™ system and GENE-UP Quant *Salmonella*; prevalence was determined using BAX detection and GENE-UP Detection. Counts were compared between location and between methods. Counts were transformed to log CFU/sample.

Results: There was a significant difference between methods at rehang location (P= 0.007), GENE-UP Quant with a mean of 0.87±0.24 log CFU/sample and SalQuant with a mean of 0.79±0.22 log CFU/sample, post pick location (P = 0.002), GENE-UP Quant with a mean of 1.21 ± 0.29 log CFU/sample and SalQuant with a mean of 0.68±0.17 log CFU/sample and pre chill location (P= 0.028), GENE-UP Quant with a mean of 0.30 ± 0.00 log CFU/sample and SalQuant with a mean of 0.18±0.35 log CFU/sample. At post chill, wings pre intervention and wings post intervention there was no significant difference found (P<0.001).

Significance: In plant bio-mapping studies are important to establish statistical process control limits that will serve facilities to improve efficacy of physical and chemical interventions schemes and target specific stages with different concentrations, for this the industry needs accurate and fast methods to generate this data.

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P1-102 Quantitative Bio-Mapping of *Salmonella* in a Commercial Poultry Processing Facility Using GENE-UP® Detection and GENE-UP® Quant *Salmonella* system to Establish Statistical Process Control Parameters and Implement Risk-Based Food Safety Management Decisions

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Introduction: Pathogen control strategies in poultry processing facilities requires sound data on pathogen loads in addition to prevalence at different stages of processing for risk-based decision-making to assist processors in compliance with regulatory performance standards.

Purpose: To develop in-plant bio-mapping of *Salmonella* prevalence and loads in a commercial poultry processing facility using GENE-UP® Quant system.

Methods: A commercial broiler processing facility was sampled during a 10-week period. Ten different locations were sampled: Live Receiving (LR), Rehang (R), Post Evisceration (PE), Pre chill (PRE), Normal Post chill (POST), Neutralized BPW Post Chill (NPOST) were sampled by the whole carcass rinse; and parts: Wings (W), Treated Wings (TW), Tenders (T) and Treated Tenders (TT) were sampled in 4 lb part rinsates. At each location, five samples were taken per repetition. A total of 50 samples were taken for each process location. *Salmonella* counts and prevalence were determined using the GENE-UP® Quant *Salmonella* system and GENE-UP® detection, respectively. Counts were transformed to Log CFU/sample for statistical analysis using R.

Results: LR sampling point had the most *Salmonella* counts with a mean of 2.94 Log CFU/sample followed by R and PE with a mean of 1.51 log CFU/sample and 1.36 log CFU/sample, respectively. PRE counts were significantly reduced on average by 1.17 log CFU/sample. Among parts, W had the higher counts with an average of 0.27 log CFU/sample. After identifying the prevalence in each process point, PE points had the higher prevalence with 95.55%, and W had the higher prevalence with 55.55%. A significant reduction in *Salmonella* prevalence was achieved in PC samples with 15.55% and TW with 11.11%.

Significance: In plant bio-mapping studies considering the quantification of *Salmonella* are important to establish risk-based statistical process control limits that will serve facilities to improve efficacy of physical and chemical interventions schemes and target specific stages for overall process improvement.

P1-103 A Novel Real Time PCR Based Risk Assessment Tool for Enteric Pathogen Indicator Organisms

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Introduction: Pathogen positive results can put food producers at significant regulatory, legal, and/or political risk. As a result, many avoid pathogen testing altogether or only do the minimum required by regulation or customer requirement. Some supplement with risk indicator methods that do not give a pathogen result but are specific for targets that include pathogens and non-pathogenic bacteria. Unfortunately, only a few risk assessment tools are currently available commercially. Eurofins GeneScan has recently developed a new tool named Enteric Pathogen Risk Indicator (EPRI). This real time PCR assay detects common gene targets associated with enteric pathogens along with some closely related non-pathogenic enteric bacteria. Importantly, EPRI presumptives can be tested directly using a fully validated pathogen specific method if desired.

Purpose: To demonstrate proof of concept of the EPRI tool with real-world samples by comparing against a validated pathogen method and culture.

Methods: Contaminated samples (Baby leafy greens, n=12) were weighed (375 G) and enriched (1:5 ratio mTSB, 20h @ 41.5°C). Following incubation, aliquots were tested by EPRI and BAC*Gene* STEC Mplex real time PCR methods, and FDA BAM STEC cultural procedures.

Results: One of twelve samples was presumptive for STEC (*stx*/*eae* pos) by the STEC multiplex method. This same sample was presumptive by EPRI. The organism could not be recovered by culture. Results agreed with those obtained by our collaborator who provided the samples.

Significance: The EPRI risk assessment tool was demonstrated to have at least equivalent performance to a pathogen specific method, and could represent a new option for food producers interested in a way to evaluate pathogen risk without testing for pathogens directly. After testing using this tool, producers have the option to test for pathogens from the same enrichment using a validated pathway.

P1-104 Co-Enrichment of *Salmonella* and Stec in Produce Matrices Prior to PCR Detection

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Introduction: Produce is routinely tested for *Salmonella* and STEC, but most commercial test kits require different enrichment conditions for the two pathogens, necessitating two samples and two separate workflows.

Purpose: To validate co-enrichment of *Salmonella* and STEC using enrichment conditions specified by Gold Standard Diagnostics' BAC*Gene* STEC Multiplex PCR kit.

Methods: Produce samples (375g) were artificially contaminated with *Salmonella* and/or STEC, and diluted 1:10 (mixed baby greens, parsley and cilantro mix, baby kale) or 1:5 (romaine lettuce and mixed leafy greens) using mTSB (without novobiocin). Samples were enriched at 41.5°C, and samples pulled at 10 and 24 hours, lysed, and tested for *Salmonella* and STEC by PCR, according to kit instructions. Additionally, asymmetric co-enrichments were performed with *Salmonella* inoculated at 1-10 CFU and STEC spiked at ~10^5 CFU to ensure that *Salmonella* would still be detectable despite a high background of STEC.

Results: Mixed baby greens, parsley and cilantro, and baby kale were all inoculated at a high spike level of both STEC (6.9 CFU/375g of *E. coli* O157) and *Salmonella* (7.4 CFU/375g of *S. enterica* ser. Senftenberg) and all samples tested positive by PCR at 10 hours (n=7 samples per matrix). In follow up studies with romaine and mixed greens (n=11 samples per matrix), *S. enterica* ser. Newport spiked at a fractional level either with or without STEC at high levels yielded PCR results at 10 and 24 hours that were in agreement with culture.

Significance: Co-enrichment of *Salmonella* and STEC in mTSB for PCR testing will reduce produce sample amounts and streamline laboratory workflow and costs.

P1-105 Evaluation of Assurance® GDS for *E. coli* O157:H7 Tq, Gds MPX for Top 7 STEC, and GDS for EHEC ID for *E. coli* O157:H7 Real Real-Time PCR Assays for the Detection of *E. coli* O157:H7 in Raw Meats, Carcass Cloths, and Raw Vegetables

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Introduction: Assurance® GDS is a molecular detection system, where the target pathogen is concentrated by immunomagnetic separation post-enrichment in mEHEC®.

Purpose: Assurance® GDS for *E. coli* O157:H7 Tq and GDS MPX for Top 7 STEC (primary STEC assays) were compared to the Canadian reference method (MFHPB-10) for the detection of *E.coli* O157:H7 in carcass cloths, raw meats, and raw vegetables. A secondary assay, GDS for EHEC ID for *E. coli* O157:H7 was applied to the same test portions to confirm the toxigenic status of presumptive positive results.

Methods: Using unpaired samples, the performance of the alternative methods were compared to MFHPB-10 (following Health Canada guidelines). Raw meat (frozen state, processed and unprocessed meats, 375 g), carcass cloths and raw vegetables (65 g and 375 g) were inoculated at three levels: 40 replicates at level (L_1) to target fractional positives (25-75%), 20 at level (L_2; 10 times L_1) and 5 un-inoculated samples. All enriched samples were incubated at 42˚C and tested concurrently by both primary assays at 8 h and 18 h, except for frozen state meats and raw vegetables (10 h and 18 h). The secondary assay was applied two hours post primary enrichment test-points. All analytical outcomes were culture confirmed by the reference method.

Results: From the 780 unpaired samples, a probability of detection (POD) statistical model determined the alternative methods exceeded the following criteria established by Microbiological Methods Committee (Canada), obtaining a relative sensitivity of ≥98%, relative specificity of ≥90.4%, false positive rate of <9.6%, false negative rate of <2% and a test efficacy of ≥94%.

Significance: The primary STEC assays are suitable methods for detecting *E. coli* O157:H7 in carcass cloths, raw meats and raw vegetables, reducing presumptive positive reporting times. A secondary assay could reduce the loss of product that otherwise would destroy unconfirmed presumptive positive adulterated lots.

P1-106 Evaluation of a Commerical Real Time PCR Assay Performance with Various Spices

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Introduction: Contamination of spices and seasoning blends with *Salmonella* remains a public health concern. Many spices contain antimicrobial components and/or assay inhibitors, which challenge pathogen detection methods, especially those using PCR for detection. Hence, test kit manufacturers often omit spices when validating methods and there is a paucity of spice validation data in comparison with other commonly tested food matrices.

Purpose: To evaluate the performance of a commercial real-time PCR *Salmonella* detection method with spice matrices having antimicrobial and PCR inhibitory properties and to determine if any method modifications are required to obtain acceptable results.

Methods: Ground black pepper, roasted garlic powder, dried oregano, dried clove, and basil were weighed and prepared following FDA BAM protocols. Duplicate samples of each were inoculated with ~10 CFU of *Salmonella* Typhimurium and tested using Gold Standard Diagnostics' BAC*Gene Salmonella* method following the manufacturer's protocol, both unmodified and with a BHI regrow step prior to lysis.

Results: All samples except dried basil yielded presumptive results when tested either with or without a BHI regrow. Post enrichment, target Cq values for all matrices were similar and quite low (20.7 avg. Cq, 0.4 SD) suggesting growth was robust. Dried basil showed significant PCR inhibition as evidenced by no amplification of the internal positive control. A 3h BHI regrow step post primary enrichment, eliminated the inhibition and presumptive results were obtained with low Cq values similar to what were observed with the non PCR inhibitory matrices.

Significance: The method reliably detected *Salmonella* in most spices tested without modification of the standard protocol, and in dried basil after a short BHI regrow step. Importantly, the total incubation time for the enrichments was not changed and all results were obtained in 24 hours or less.

P1-107 Colony Confirmation by Real-Time PCR for *Salmonella* and STX1 and/or STX 2 Positive *Escherichia coli*

Patricia Rule, Samoa Asigau, Patrick Bird, Jada Jackson, Nikki Taylor, TrudyAnn Plummer, Michelle Keener, Deborah Briese, John Mills, Vikrant Dutta and Ron Johnson

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***Introduction**:* In the fast-growing cannabis industry regulatory compliance is becoming increasingly standardized with the need for *Salmonella* and pathogenic *Escherichia coli* detection at the top of cannabis product quality and safety programs. The GENE-UP® PRO™ STEC/*Salmonella* utilizes multiplex PCR for the simultaneous detection of *Salmonella* and/or pathogenic (stx1 and/or stx2 positive) *E. coli.* It already has AOAC PTM status (License # 092110) for a variety of cannabis products (flower, extract, oils, edibles) and environmental samples. However, organism culture confirmation is not often pursued as it is tedious and time consuming and requires additional expertise that is not yet well established in the cannabis industry.

Purpose: Therefore, a study was conducted for real time PCR confirmation from organism growth from selective and non-selective agars that could offer a same day identification of an isolated colony direct from plate.

***Methods**:* 150 *Salmonella* species were grown overnight at 35 °C on *Salmonella* agars (XLD and ASAP™) agars to establish inclusivity for *Salmonella* and 100 pathogenic *E. coli* were grown on selective CHROMID™ EHEC agar for STEC inclusivity. 100 Gram-negative organisms were grown overnight on Blood Agar to establish exclusivity.

***Results**:* All 150 *Salmonella* target isolates correctly reported *Salmonella* with PRO STEC/*Salmonella* whether grown on ASAP™ or XLD. All 100 pathogenic *E. coli* on EHEC agar were positive for STEC with the GENE-UP PRO STEC/*Salmonella* assay. All 100 Gram-negative strains grown on non-selective Blood agar reported negative. Inclusivity and exclusivity from the isolated colonies was in 100% agreement with the expected results.

Significance: This data supports real time PCR colony confirmation as a rapid identification method that would provide the cannabis industry with valuable information without the need for challenging and time-consuming biochemical isolate confirmation which would reduce holding time but more importantly provide a proper identification for early intervention and action.

P1-108 Direct Colony Confirmation by Real-Time PCR Using GENE-UP® *Salmonella*

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***Introduction**:* Large outbreaks of illness caused by *Salmonella*-contaminated products continue to make headlines. With more than 28 *Salmonella* related recalls in 2022, the screening and detection of this pathogen remains forefront in all food safety and quality programs. The GENE-UP® *Salmonella* (SLM) real-time PCR method has both AFNOR (BIO 12/38-06/16) and AOAC (OMA 2020.02) approvals for a variety of food, petfood and environmental samples in varying sizes, enrichment media and temperatures. However, culture confirmation for presumptive screens remains a limiting step to any pathogen safety program as final identification steps are still tied to the traditional growth-based biochemical analysis.

***Purpose**:* A study was conducted for real time PCR confirmation from isolated colonies on select *Salmonella* positive samples following 2 protocols. One direct from agars and second from isolates reinoculated into enrichments once diluted to 100x the LOD.

***Methods**:* 150 *Salmonella* species were grown overnight in BPW or on ASAP and XLD selective agars to establish inclusivity. An additional 100 non-*Salmonella* strains were grown overnight on Blood Agar to establish exclusivity. Individual colonies were isolated with a sterile loop and inoculated into 0.2 mL of Molecular water. Isolated colonies were vortexed, lysed and then evaluated on GENE-UP SLM.

***Results**:* All 150 *Salmonella* target isolates were correctly reported positive with GENE-UP SLM whether grown on ASAP or XLD agar plates. All 100 non-*Salmonella* species grown on Blood agar were reported negative. Therefore, inclusivity and exclusivity from the isolated colonies was in 100% agreement with the expected results.

***Significance**:* This data supports real time PCR colony confirmation as a rapid identification method for *Salmonella* that would provide food producers and manufacturers valuable early information which would allow earlier intervention to avoid potential outbreaks.

Poster

P1-109 The Review of Multiplex Real Time PCR for the Confirmation of Yeast and Bacteria from Fruit Flavored Water Post Growth in BACT/ALERT iLYM Culture Bottles

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Introduction: The high acid beverage industry remains highly innovative regularly creating new products to meet consumer demands. Early detection of spoilage is of primary importance but once detected comes the challenge of culture confirmation. Each spoilage organism has specific growth requirements which means multiple isolation media and temperatures.

Purpose*:* A study was conducted with a Fruit Flavored Water spiked with low levels of potential spoilage organisms. The study followed three aspects. Did the organisms survive and grow in the product. If so, was it detected in the BACT/ALERT® (BTA) iLYM bottle and finally could multiplex PCR be used to identify the surviving organism.

Methods*:* Fruit Flavored Water (8.45 oz) was spiked in duplicate with either *Candida tropicalis* (17 CFU), *Lactobacillus fermentum* (4 CFU), *Bacillus coagulans* (6 CFU) or *Bacillus subitilis* (9 CFU). Due to the lower nutritional value of the product, packages were pre-incubated for 4 days. Post pre-Incubation, 5 mL was inoculated (x2) into BTA iLYM and monitored for 2 days. 25 mL of each positive iLYM bottle was evaluated (x2) using GENE-UP® PRO™ BEVERAGE to confirm the surviving organism.

Results*: Candida*, *Lactobacillus* and *Bacillus coagulans* grew directly in the Fruited Water as well as in all BTA iLYM bottles. *C. tropicalis* detected in < 4 hours and *Lactobacillus* in < 8 hours in BACT/ALERT while *Bacillus coagulans* took 1.5 days. GENE-UP® PRO™ BEVERAGE correctly confirmed to genus-level the spiked organism in all samples. *Bacillus subtilis* did not grow in the Fruit Flavored Water.

Significance*:* The multiplex PCR assay targeting *Candida*, *Pichia*, *Dekkera*, *Sachharomyces cerevisiae* var. *diastaticus*, *Lactobacillus*, *Pediococcus, Alicyclobacillus, Acetobacter, Gluconobacter,* or *Bacillus* could provide genus-level identification the same day the sample is flagged positive by the BTA system without the need for the tedious and challenging task of plating allowing for early intervention and mitigation in the manufacturing process.

P1-110 Evaluation of Accuracy and Efficiency for Novel Automatic Colony Counting System for Ready-to-Use Culture Media, Easy Plate™

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Introduction: Ready-to-use (RTU) culture media enhances food safety and productivity because of its quickness, compactness, simplicity, and visibility of colonies compared to conventional agar media. Since colony counting requires skilled technicians and is time-consuming, automated colony counting systems are used for better efficiency.

Purpose: To confirm the rapidity and accuracy of the novel "Colony Counting System for Easy Plate (CCS)" - a plate reading technology based on a general scanner with auto document feeder and analysis software.

Methods: The CCS consists of a scanner (Brother Industry, LTD., ADS-4300N) and counting software based on object detection using machine learning. Ten food samples were diluted and inoculated onto RTU-culture media, Easy Plate AC (Kikkoman Biochemifa Company) for aerobic bacteria count (AC) and Easy Plate CC for coliform bacteria count (CC) at three contamination levels and incubated according to the respective instructions for use. Differences of bacterial counts and measurement time between conventional manual methods and CCS method were evaluated.

Results: The CCS method took 5.0 to 6.4 seconds/sheet regardless of contamination level. On the other hand, conventional manual methods took 13.8 to 44.5 seconds/sheet at low contamination levels ranging from 10 to 50 CFU/sheet, the level at which manual counting methods were shown to be most rapid. The correlation coefficients for bacterial counts between manual counting and the CCS method were greater than 0.98 for both AC and CC. These results indicated that 77% of analysis time could be saved by the use of the CCS method with counting accuracy comparable to manual methods.

Significance: It was demonstrated that this system provides automated colony counting with rapidity and accuracy. An automated system can also be expected to reduce human error. Therefore, the combination of RTU-culture media and an automated system can save time and reduce costs of microbiological testing while maintaining accuracy.

P1-111 Verification of Ready-to-Eat (RTE) and Raw Fermented Products for the Detection of *Salmonella* and *Listeria* Using the GENE-UP® *Salmonella* (SLM), *Listeria monocytogenes* (LMO), and *Listeria* spp. (LIS) Assays

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Introduction: Ready-to-eat (RTE) and raw meat products can be contaminated by pathogenic bacteria such as *Listeria monocytogenes* and *Salmonella* spp. and have been implicated in multiple foodborne outbreaks. According to the U.S. Department of Agriculture Food Safety Inspection Service (FSIS), any level of *Listeria monocytogenes* or *Salmonella* detected in RTE product is considered adulterated, and inadequate cooking and cross-contamination are concerns for raw product, therefore it is critical to have appropriate testing measures in place for both types of fermented foods.

Purpose: To verify the performance of the candidate method real-time PCR assays in association with the confirmation methods outlined in the USDA FSIS MLG 4.12 (*Salmonella*) and 8.13 (*Listeria monocytogenes*) on various fermented food products.

Methods: This study evaluated replicates of RTE or raw salami, Italian sausage and chorizo. For *Salmonella*, 325g test portions were evaluated and for Listeria 125g test portions were evaluated. A total of 15 replicates (five of each food type) for each target were artificially inoculated with a low level of target organism and six replicates (two of each food type) were used as uninoculated controls. Alternate and reference confirmation protocols were performed on every sample regardless of screening result.

Results: All of the low level replicates for each target were positively detected by the corresponding PCR assay and were in 100% agreement with both the alternate and reference confirmation results. All of the uninoculated controls were negative for the target organisms used for the artificial contamination, however one food type was found to have natural contamination of *Listeria welshimeri*.

Significance: These data indicate that the GENE-UP SLM, LMO and LIS assays are sufficient for use in the detection of these potential pathogens from various fermented food products.

P1-112 Validation of the GENE-UP enviroPRO™ Assay with *Biomérieux* Universal Enrichment Media: *AOAC Performance Tested MethodSM* 061801

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Introduction: Salmonellosis has mostly been associated with outbreaks due to foods of animal origin. There are an estimated 1.35 million cases of Salmonellosis each year in the United States, the majority of which can be attributed to food sources. Listeriosis is a serious infection that is most dangerous for pregnant women and their newborns, the elderly, or immunocompromised individuals and has a reported 1,600 cases each year in the United States. The GENE-UP enviroPRO assay is a real-time PCR assay for the simultaneous presumptive detection of *Listeria* and *Salmonella* species from environmental samples and food matrices.

Poster

Purpose: Conduct a Level 3 modification study to allow for the detection of *Listeria* and *Salmonella* from a single enrichment, Universal Enrichment media and incorporate a step to eliminate DNA detection from non-viable cells. The candidate method was also validated as confirmation assay from colonies on select agar plates.

Methods: The candidate assay was evaluated in a matrix study against the FDA BAM Chapter 5 *Salmonella* and Chapter 10 *Listeria monocytogenes* assays for three matrices: environmental surface sponges from stainless steel, sealed concrete and plastic. Testing included the BACTIVAB PMAxx DNA removal protocol. Inclusivity and exclusivity of the new enrichment and from isolated colonies on select agars was performed.

Results: In the matrix study, the candidate assay demonstrated comparable results to the reference method with and without the BACTIVAB PMAxx protocol. In the inclusivity and exclusivity study, the target isolates were correctly identified by the candidate method and all non-target isolates were correctly excluded from the new enrichment media as well as from isolated colonies on agar plates.

Significance: The data from this study supports the product claim that the GENE-UP enviroPRO™ assay can simultaneously detect *Salmonella* and *Listeria* species in environmental sponges from stainless steel, sealed concrete and plastic when enriched using Universal Enrichment media.

P1-113 Multi-Laboratory Validation Study of a Real-Time PCR Method for Detection of *Salmonella* in Frozen Fish

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Introduction: The FDA BAM *Salmonella* culture method is commonly used for detection and confirmation of the pathogen when food outbreaks occur but takes four days to produce presumptive positive results.

Purpose: To expedite faster *Salmonella* detection, the FDA developed a real-time PCR (qPCR) method able to detect the pathogen within 24 hours using the ABI 7500 FAST PCR system and evaluated the method in previous single laboratory validation (SLV) studies. The method's performance and reproducibility were further confirmed in this Multi-Laboratory Validation (MLV) study in frozen fish.

Methods: Fourteen laboratories from FDA ORA and state public health departments participated in the study. Each participating laboratory analyzed 25 samples, which included 8 uninoculated, 8 low level, and 8 high level test samples, along with 1 uninoculated sample for total aerobic plate count. Test samples were analyzed using the BAM *Salmonella* culture method as the reference method and the qPCR method as the alternative method. Statistical analysis was performed per ISO16140-2:2016 (Microbiology of the Food Chain-Method Validation-Part 2).

Results: The low inoculation level resulted in a positive rate of 38.5% for the qPCR method and 40.3% for the BAM culture method, both within the FDA Microbiological Method Validation Guideline level of 50%±25%. The high inoculation level resulted in a positive rate of 99% for both methods. Statistical analysis showed that the relative level of detection (RLOD) was 1.0, suggesting that the qPCR method and the culture method had statistically similar detection rates ($P>0.05$).

Significance: This MLV study further affirms that the qPCR method has highly reproducible results among laboratories and is sufficiently sensitive and specific for detecting *Salmonella* in frozen fish as a rapid screening method. Previously validated using baby spinach and now validated in frozen fish, this molecular method will serve as an important tool in outbreak investigation and regulatory research.

P1-114 Detection of *Salmonella* Typhimurium in Frozen Chicken Cordon Bleu across Multiple Laboratories Utilizing Varying Methods

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Introduction: An inter-laboratory comparison (ILC) exercise was conducted to detect *Salmonella* in frozen not-ready-to-eat chicken cordon bleu (CCB) artificially contaminated with different levels of *Salmonella typhimurium*.

Purpose: To evaluate Rates of Detection (ROD) of *Salmonella* in CCB at different inoculation levels in 25 g and 325 g test portions utilizing different methods.

Methods: CCB Samples for ILC were inoculated and verified for homogeneity and stability according to ISO 16140. Labs received either 8x 25g test portions with 0, 10, 30 or 300 CFU of *Salmonella*, or 8x 325g test portions with 0, 500, 1,000 or 10,000 CFU of *Salmonella*. Each level was tested in duplicate. Most labs analyzed samples using USDA, BAM or AOAC methods.

Results: Among the 26 labs testing 25g portions - 10 reported expected RODs (0% for 0 CFU, 100% for other samples); 6 reported 50% and 100% ROD for 30 and 300 CFU respectively; 2 reported between 0%, 100% and 100% RODs for 10, 30 and 300 CFUs respectively. One lab reported no detection and 3 reported 50% or 100% ROD for 300 CFU. Four labs reported RODs that did not increase with inoculation levels, resulting in higher level of detection (LOD) than labs with ideal or almost ideal detection. Among the 6 labs testing 325g portions, 4 reported expected RODs; 1 reported 50% ROD for 1000 and 10,000 CFU and 1 reported positive results in both uninoculated samples and 50% ROD for high samples.

Significance: CCB is a multi-component matrix with raw chicken breast, ham, cheese, flour and spices. There are significant differences between the RODs and the LODs of the labs, likely related to the complex matrix and methodology used. This ILC demonstrates that such multi-component foods present unique challenges that may affect the recovery of *Salmonella*, requiring labs verification of methods for complex matrices.

Poster

P1-115 Matrix Validation of 25 Ml Apple Juice for the Detection of *E. coli* O157:H7 and *Salmonella* Using the Hygiena® BAX® System

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Introduction: Fruit juices typically have a low pH, ranging from 2.5 to 4.0. This acidic level serves as an important barrier to microbial growth. Some pathogens, however, can adapt to the surrounding environment and cause juices to become unsafe for consumption.

Purpose: This study was designed to validate the use of two real-time PCR assays for the detection of *E. coli* O157:H7 and *Salmonella* in 25 ml of apple juice.

Methods: Apple juice was inoculated with both *E. coli* O157:H7 and *Salmonella* at two contamination levels: either a low level of 1 CFU/25 ml or a high level of 10 CFU/25 ml. Test portions were stabilized at 4°C for approximately 72 hours and then divided into 3 sets of 30 unpaired samples. Test method samples were enriched in BPW, incubated at 37 °C for 12-24 hours and analyzed by real-time PCR. Reference samples were enriched and confirmed according to the procedures in the FDA BAM Chapter 4A for *E. coli* and Chapter 5 for *Salmonella*.

Results: For low inoculum samples enriched in BPW, *E. coli* O157:H7 was detected in 4/20 samples, and *Salmonella* was detected in 6/20 of the same samples. All high inoculum samples were detected by real-time PCR, and all results were identical to culture for both organisms. Compared to the reference method, POD analysis indicated no significant difference for either organism.

Significance: Overall, the results between the BAX® System Real-Time PCR assays and the FDA BAM reference methods were indistinguishable for the detection of *E. coli* O157:H7 and *Salmonella* in 25 ml of apple juice.

P1-116 Evaluation of the Hygiena® BAX® System PCR Assays for the Detection of *Salmonella* from Pooled Environmental Sponges

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Introduction: In pathogen environmental monitoring programs, the sampling frequency and numbers of samples vary depending on the potential risk of contamination. For high-risk product types and production processes, this could mean a substantial amount of testing.

Purpose: To reduce the laboratory workload and cost, some processors will combine multiple enriched samples creating a pooled test portion for analysis. The purpose of this study was to evaluate the efficacy of real-time and standard PCR assays for *Salmonella* from pooled environmental sponges.

Methods: Unpaired stainless-steel surfaces were inoculated with *Salmonella* and a competitive microorganism creating 20 low-level samples, 5 high-level samples, and 5 controls for each of 2 methods being compared. After the inoculum was dry, surfaces were swabbed and enriched either using the test method (BPW) or FDA BAM Chapter 5 (LB). All enrichments were tested by PCR as individual and pooled samples (1 inoculated sample combined with 4 uninoculated controls) and culture confirmed.

Results: PCR results for individual and pooled sponges had 100% sensitivity and 100% specificity for both the test method enrichment and the reference method. All presumptive PCR results were identical to culture results. POD analysis for the low-level samples indicated a significant difference between BPW (0.75) and LB (0.35) sponges, as BPW sponges recovered significantly more positives.

Significance: Pooled sponges performed equivalent to single, individual test sponges using the BAX® System PCR for the detection of *Salmonella*.

P1-117 Digital PCR Assay for the Specific Detection and Estimation of *Salmonella* Contamination Levels in Poultry Rinse

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❖ ***Developing Scientist Entrant***

Introduction: Strains of *Salmonella* are known to contaminate poultry products and they are a frequent cause of human infections. Currently, regulatory agencies and other food testing laboratories rely on a combination of enrichment, real-time PCR, and culture-based methods for specific detection and confirmation of *Salmonella* in food samples. One limitation of these methods is their inability to quantify or estimate the *Salmonella* load in samples.

Purpose: The aim of the study was to standardize a digital PCR (dPCR) assay for the detection and estimation of *Salmonella* contamination levels in poultry rinses.

Methods: Pure culture *Salmonella* Typhimurium strain (ATCC 14028) was cultured overnight and enumerated using plate count agar. Four hundred milliliters of phosphate buffer saline was used to prepare whole carcass chicken rinse (WCCR). Plate counts were used to calculating the inoculation volume and WCCR samples were inoculated at 1 – 4 log CFU/30 mL. Inoculated samples were cold-stressed by storing samples at 4°C for 48 h. These samples were enriched using 15ml of 3× neutralizing buffered peptone water with novobiocin and sodium pyruvate. Samples were enriched for five hours at 35 °C, DNA from 200 µl enrichments were isolated, and undiluted DNA samples were directly used for dPCR assay. Primer and probe targeting the *Salmonella invA* gene were used with QIAcuity Probe PCR Kit and data was analyzed using QIAcuity Software Suite.

Results: The dPCR assay showed high tolerance for DNA and PCR inhibitors. The dPCR assay showed no PCR reaction inhibition up to 5 micrograms of crude DNA extract. The assays accurately detected all cold-stressed *Salmonella* in inoculated WCCR samples following a 5 h enrichment. The dPCR value (copies/µl) when converted to $\log_{10}$, accurately estimated the inoculated *Salmonella* levels.

Significance: This dPCR assay is a robust method for the detection and estimation of *Salmonella* concentration in contaminated food samples.

P1-118 Development of Digital Polymerase Chain Reaction for Detection of Non-Bacterial Pathogens in Environmental Monitoring Samples

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❖ ***Developing Scientist Entrant***

Introduction: Pathogen detection in soil, water, swab, and food samples is critical to surveillance and outbreaks. Multiple methods exist for detection of bacteria, while detection of *Cyclospora*, hepatitis A virus (HAV), and norovirus (NoV) remains difficult.

Purpose: Methods were developed for the use of digital polymerase chain reaction (dPCR) to detect non-bacterial foodborne pathogens.

Methods: Soil samples (30g) were inoculated with *Eimeria tenella* oocysts ($5x10^4$) and HAV ($1x10^3$), mixed with 1XPBS plus 3% beef extract (120mL) and massaged for 2 minutes in filtered/non-filtered Whirl-pak bags. Samples (60mL) were subjected to ultrafiltration and centrifuged at 3,000xg for 45mins at 20ºC. Concentrate was collected and extracted using either the Qiagen DNeasy PowerSoil or RNeasy PowerMicrobiome kit. Samples were subjected to dPCR. After determining recovery efficiency, farm soil samples across 2 NE US states (n=96) were assessed for *C. cayetanensis*, HAV, NoV GI & GII.

Results: *E. tenella* oocysts as a protozoa control, were recovered at an average of 83.9copies/g, with an absolute quantification value of 124.5-*partitions*. Oocyst detection by dPCR from filtered soil samples was 9.38copies/g, and 9.0copies/g in unfiltered samples, an insignificant difference (*p*=0.95). Absolute quantification values by dPCR were 12.7 and 12.6-*partitions* for filtered and unfiltered samples, approximately a 1-log reduction from the absolute quantification value found in the process control (124.5-*partitions*). Extraction/sampling in conjunction with dPCR, is sufficient for detecting low levels of oocysts in soil. In inoculated HAV-control samples, HAV was recovered at an average rate of $1.2x10^5$copies/g of soil with an absolute quantification value of $2.5x10^5$-*partitions*. Recovery was greater from filtered soil ($3.27x10^2$copies/g) versus unfiltered ($1.85x10^2$copies/g)($p<0.0001$). The absolute quantification values of HAV were $4.48x10^2$ and $2.51x10^2$-*partitions* for filtered and unfiltered, respectively. None of the 96 samples were positive for *Cyclopsora* or HAV.

Significance: The sensitivity of dPCR yields accurate absolute pathogen detection and likely rapid detection of challenging organisms across matrices.

P1-119 Evaluation of a PCR Workflow for the Detection of *Salmonella* from Pooled Chocolate Ingredients

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Introduction: In recent years there have been several *Salmonella* outbreaks linked to nuts, dry fruit, and chocolate, which have led to hospitalisations. A reliable detection method is valuable to ensure that chocolate and low water activity chocolate ingredients are safe to eat; pooling methods offer high throughput testing for matrices with a low incidence of *Salmonella* contamination.

Purpose: The purpose of the study was to evaluate the Thermo Fisher Scientific™ SureTect™ Salmonella species PCR workflow for the detection of *Salmonella* from chocolate ingredients after a post-enrichment pooling step.

Methods: A total of 36 spiked samples were tested, comprising whole almonds, chopped hazelnuts, hazelnut paste and raisins. Two-hundred-and-fifty gram samples were artificially contaminated using seeding and heat injury methods. Per data point, ten samples were pooled (one part contaminated with nine parts non-contaminated) and tested using the PCR workflow and ISO 6579-1 reference method.

Results: The PCR assay was able to successfully detect *Salmonella* from all of the low water activity matrices, following pooling, after a 20 hour enrichment. The results were in complete agreement with the ISO reference method.

Poster

Significance: The data shows that the PCR workflow is a reliable method for the detection of *Salmonella* from pooled chocolate ingredients including nuts and dried fruit.

P1-120 Performance Equivalency and Stability Analysis of Handling Improvements of the Thermo Scientific Suretect Workflow

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Introduction: Real-time PCR detection of foodborne pathogens from food and environmental surfaces is a widely used principle in the food safety industry. The Thermo Scientific™ SureTect™ workflow is a real-time PCR detection method for a number of foodborne pathogens. The importance of time to result and efficiency in process is a critical factor in food pathogen testing, considering this, a number of handling improvements were identified and analysed for equivalency and stability.

Purpose: Analyse improvements to ensure equivalency in performance whilst improving efficiency. Improvements include blue dye indicator moving from one reagent to another providing a visual indicator whilst pipetting, addition of a pierceable lysis seal, rigid lysis/PCR plates with color coding and orientation markers. Improved capping, de-capping and cutting tools are also available but were not analysed due to no impact on performance/stability.

Methods: Where applicable, improvements were analysed for stability, performance equivalence and enzymatic activity between the original and new formats. Studies were designed in accordance with the manufacturing site quality system (ISO 13485:2016 certified) with added enhancements where required. A representative range of assays and matrices were included. Acceptance criteria was based on the current variation between SureTect Assays (Ct value ±1.5 and dRn at ±50%).

Results: The stability testing showed equivalent performance across all time points (accelerated and real-time) for all improvements. Performance testing showed equivalent performance for all improvements. Enzymatic analysis for the blue dye relocation showed equivalent performance. The improvements were reviewed and approved by *AOAC Performance Tested Methods*℠, *AOAC Official Method of Analysis*℠, NF VALIDATION by AFNOR Certification and MicroVal Certification.

Significance: The SureTect workflow improvements offer increased efficiency and a reduced handling step with no impact to performance. New capping, de-capping and cutting tools have also been added.

P1-121 A Case Study of *Salmonella* Quantitation and Serotyping in Poultry Production Samples

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Introduction: Over 23% of the 1.35 million human *Salmonella* infections in the US are attributed to poultry consumption. The USDA has proposed a new regulatory framework, including enhancing process control monitoring, with the aim of preventing illness by lowering *Salmonella* contamination rates in finished poultry products. Quantification of *Salmonella* throughout production, with a focus on pathogenic serovars, allows for more detailed and useful data to be gathered and supports determination of hygiene control measure impact on product contamination.

Purpose: This case study evaluated performance of the Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis PCR workflow for assessment of contamination level of *Salmonella* and selected serotypes in poultry production matrices.

Methods: The PCR workflow utilises a short selective enrichment followed by qPCR to estimate the pre-enrichment contamination level, expressed as CFU/sample. Sixteen naturally contaminated hot re-hang rinse samples were analysed using both the PCR quantitative workflow and a MPN method in an unpaired study.

Results: When contamination level estimates were generated by the PCR workflow across 16 hot re-hang rinse samples, 100% were within 1 log and 87.50% within 0.5 log of the MPN method for *Salmonella* species. Fifteen samples tested positive for *Salmonella* ser. Enteritidis, achieving 86.67% and 73.33% of estimates within 1 and 0.5 log of the MPN method respectively. Data collected for *Salmonella* ser. Typhimurium was limited due to low prevalence within the samples. All contamination estimates were automatically calculated using the RapidFinder Analysis software without need for user intervention.

Significance: The PCR workflow produced comparable results to the MPN workflow from re-hang samples, with a shorter time to result. Additionally, the PCR workflow was able to identify and quantify *Salmonella* Enteritidis and *Salmonella* species in a single PCR test without additional testing.

P1-122 Effect of Pooling on Molecular Detection of *Salmonella* and *Listeria monocytogenes* on Raw and Cooked Shrimp

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Introduction: Shrimp can be a vehicle for foodborne pathogens such as *Salmonella* and *Listeria monocytogenes* which can persist in aquatic environments. For the shrimp industry, rapid and cost-effective tools to detect these pathogens can help prevent the release of contaminated products into the market.

Purpose: This study evaluated the effect of post-enrichment pooling on detection of *Salmonella* and *L. monocytogenes* in headless raw and cooked shrimp using loop-mediated-isothermal-amplification (LAMP).

Methods: Shrimp samples were obtained directly from the procesing site. Twenty-five gram test portions were spiked with 5, 1, and <1 CFU of *Salmonella* Paratyphi A ATCC 9150 or *L. monocytogenes* ATCC 19112 and 100 times more of an interferent microorganism. For each shrimp type, one out of three samples were spiked with each microorganism at each inoculum level. Samples were incubated at 37°C for 22h and 24h in Buffered Peptone Water or Demi-Fraser Broth followed by secondary enrichment following USDA-MLG reference method. Enrichments were analyzed by LAMP individually, and also by pooling one positive and two negative enrichments. Each combination was repeated seven times for each incubation scheme. Pooled and individual enrichments were compared using Chi-Square test. Sensitivity and specificity were calculated for each condition.

Results: Statistical differences were found in *Salmonella* (X^2>3.84) in raw (24h single enrichment) and cooked shrimp (secondary enrichment) when comparing pooled and single samples. Sensitivity rate was >90% for pooling for *Salmonella* (cooked shrimp 22h and 24h single enrichment steps) and *L. monocytogenes* (raw and cooked shrimp with 22h and 24h secondary enrichment steps). Specificity rate of >90% was found for *Salmonella* (raw shrimp 24h single enrichment and 24h secondary enrichment, cooked shrimp 22h secondary enrichment) and *L. monocytogenes* (raw and cooked shrimp 22h single enrichment).

Significance: Pooling should only be used with select samples and validated incubation conditions as it may impact performance attributes such as sensitivity and specificity.

Poster

P1-123 Validation of Rapidchek® *Campylobacter* Test System for the Detection of *C. jejuni*, *C. coli*, and *C. lari* in Poultry Samples

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Introduction: *Campylobacter* is one of the leading causes of human bacterial gastroenteritis worldwide. *Campylobacter* infections are often associated with the consumption of raw milk, undercooked poultry, and contaminated water. Effective monitoring of the various control strategies implemented during poultry processing is critical for reducing *Campylobacter* in the final product.

Purpose: The RapidChek® *Campylobacter* test system was validated for the detection of *Campylobacter jejuni*, *Campylobacter coli,* and *Campylobacter lari* in raw ground chicken, chicken carcass rinse, and turkey carcass sponges.

Methods: *Campylobacter*-inoculated food samples were tested by the candidate method and the USDA/FSIS cultural reference method; Isolation and Identification of *Campylobacter jejuni/coli/lari* from Poultry Rinse, Sponge and Raw Product Samples MLG 41.04. The candidate method samples were enriched aerobically at 42ºC for 47 to 49 hours. Following aerobic enrichment, an immunochromatographic test strip is inserted into the tube containing the enrichment, developed for 20 minutes, and interpreted. The candidate method enrichments were confirmed culturally. Inclusivity studies included 50 *Campylobacter* strains comprised of *C. jejuni*, *C. coli*, *C. lari* and 30 non-target strains.

Results: A total of 80 low-level spiked samples were tested by both methods in the study. The candidate method yielded 49 presumptive positives; all presumptive results were confirmed culturally. The reference method produced a total of 41 confirmed positive results. Probability of Detection analysis demonstrated no significant differences in the number of positive samples detected by the candidate method and cultural reference method. All 50 inclusivity Campylobacter strains were detected, whereas none of the 30 exclusivity strains, including non-target *Campylobacter* spp., were detected.

Significance: The rapid detection and simplified aerobic incubation conditions of the new Campylobacter method presents a cost effective and reliable tool for monitoring the efficiency of implemented *Campylobacter* reduction steps throughout the production process.

P1-124 Evaluation of Planar Spiral Coil-Based Magnetoelastic Biosensor for Simultaneous Detection of *Salmonella* typhimurium and *Escherichia coli* O157:H7 on Fresh Produce

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Introduction: A novel planar spiral coil-based magnetoelastic (ME) biosensor, consisting of three sensors, a movable planar spiral coil, and a set of hand-held signal amplifiers, was constructed. It was combined with *Salmonella* Typhimurium (ST) and *Escherichia coli* O157:H7 (EC)-specific phages to simultaneously detect ST and EC on apple.

Purpose: The purpose of this study was to evaluate a planar spiral coil-based ME biosensor combined with phage and evaluate simultaneous detection of ST and EC.

Methods: To determine the exposure time for bacterial detection, each phage-immobilized ME sensor was placed on ST or EC contaminated apple, after which RF shift of sensor was measured 1 min interval for 30 min. After dividing the zone of planar spiral coil, RF of three ME sensor was measured simultaneously by moving sensors for the determination of appropriate distance among sensors. To evaluate the sensitivity, linearity, and detection limit, each phage-immobilized ME sensor was exposed to an apple contaminated with various concentration (2, 4, 6, and 8 log CFU/25 mm2) of bacterial mixture of ST and EC, and RF shift of each ME biosensor was measured.

Results: As the exposure time of phage-immobilized ME biosensor increased, the RF shifts of each phage-immobilized ME biosensor increased until 16 min and then maintained. The three ME biosensors produced their RF peaks without aggregation when the distance between the two sensors was more than 1.2 mm apart. As the concentration of bacterial mixture on the apple increased, each phage-immobilized ME biosensor exhibited dose-dependent manners in the RF shifts and detected its target bacteria simultaneously with a detection limit of 1.7 ± 0.4 log CFU/25 mm^2 for ST and 1.6 ± 0.3 log CFU/25 mm^2 for EC.

Significance: The planar spiral coil-based ME biosensor is a rapid, sensitive, efficient method for the simultaneous detection of foodborne pathogens.

P1-125 Poresippr: A Rapid Method for the Characterization of Shiga-Toxin Producing *E. coli* (STEC) Using Nanopore Sequencing

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Introduction: The rapid identification and characterization of priority foodborne pathogens such as Shiga-toxin producing *E. coli* (STEC) is critical for informing food-safety investigations; however, current approaches based on short-read whole-genome sequencing take days, and may fail to accurately identify Shiga toxins.

Purpose: To develop a method for rapid risk-characterization of STEC recovered from foods, using Oxford Nanopore Technologies (ONT) long-read sequencing.

Methods: A panel of 30 STEC strains were selected for this study. Long-read sequences were generated using the rapid barcoding kit (SQK-RBK004) and the Flow Cell and/or Flongle (both R9.4.1). Basecalling was conducted with MinKNOW using the fast and/or high-accuracy settings. To assess the impact of sequencing depth and basecalling algorithm on accuracy of target detection, a modelling experiment was conducted with 3 strains. Reads were subsampled (10 per coverage level: 1x, 2.5x, 5x, 7.5x, 10x) and aligned to exact match probe sequences, probes varying in length and percent identity (simulating natural variation of Shiga-toxins), and a comprehensive Shiga-toxin database. A pilot study using the MinION was conducted using live-basecalling and alignment to a custom database comprising serotype markers, virulence genes, and Shiga-toxins.

Results: Modelling demonstrated that serotype and virulence factors (including Shiga-toxin subtype) could be accurately identified in 100% of subsamples at 5x coverage using full-length probes. Targets were detected in 100% of subsamples at the lowest percent identity (85%) at 7.5x coverage, demonstrating that targets could still be detected despite variation in probe sequence. A pilot study demonstrated that virulence factors could be accurately identified within 2hrs at a similar cost to current approaches.

Significance: The PoreSippR method is easily implementable and could significantly reduce the time required to characterize STEC. It provides valuable long-read sequencing data, critical for accurate identification of full-length Shiga-toxin genes, which will contribute to more comprehensive information for risk assessment of STEC recovered from the food supply.

P1-126 Evaluation of a Rapid Technology to Detect Microbial Contamination in Ultra High Temperature Processed Plant-Based Beverages in Mexico

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Introduction: Thermal processing technology has been widely applied for food preservation. Ready to eat products, processed under Ultra High Temperature (UHT) and aseptically filled are extensively consumed. Sterilization and aseptic filling are critical steps and their efficacy must be verified by commercial sterility test. Bioluminescence can be used as a powerful tool to detect microbial contamination on an early way.

Poster

Purpose: To evaluate a rapid microbial ATP-detection method and compare with agar to evaluate commercial sterility in UHT plant-based beverages.
Methods: Three different UHT plant-based flavor beverages were evaluated: Almond, almond sugar-free and coconut. Each (960 mL) product were spiked with the next microorganism: *Bacillus subtilis (*ATCC 6633), *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (ATCC 29212) at three levels: 1-50, 50-100 and 100-500 CFU/brick. Samples were incubated at 37°C during two different time periods: 72 and 120 h. Ten samples were spiked per combination (flavor/microorganism/inoculum/incubation time). Thirty samples were used to determine pass/fail limits per flavor and incubation time and were used as negative controls.
Each sample was evaluated by the rapid microbial ATP detection method (MLSII by Neogen) and steaked on plate count agar (Aerobic growth - 48h) to detect presence/absences. Squared chi was used to determine statistical differences among methods. Sensibility and Specificity was determined.
Results: Overall were not found differences among testing methods when product is incubated 72h (x^2=0.6), sensibility and specificity rate was 79 and 96% respectively. For samples spiked with *Enterococcus faecalis* and incubated 120 h statistical differences were found; twenty-seven out ninety spiked samples cannot be confirmed by agar probably because the were on death phase. All the negative control yielded as negative by both methods.
Significance: The microbial-ATP based detection method provided accurate and faster results when compared to agar to detect microbial contamination in UHT plant-based products.

P1-127 Rapid Pathogen Classification Using Magnetic Nanoparticles and Machine Learning Applied to Near Infrared Spectroscopy Data

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❖ ***Developing Scientist Entrant***

Introduction: Rapid identification of foodborne pathogens is critical to prevent outbreaks that have significant health and financial impacts.
Purpose: While reliable protocols are available for identification of pathogens from complex food matrices, sample enrichment can be a time-consuming process and faster methods are needed.
Methods: Glycan-coated magnetic nanoparticles were used to concentrate and extract *Bacillus cereus*, *Salmonella* enteritidis, and *E. coli* from Tryptic Soy Broth following which their absorption spectra in the near-infrared region were obtained. Following data pre-processing, principal components were obtained using singular value decomposition. Supervised classification algorithms including Support Vector Machine, Linear Discriminant Analysis, and Neural Networks were used for bacteria identification.
Results: S. enteritidis was classified with a classification accuracy of >90% against both *B. cereus* and *E. coli* (n=56). *B. cereus* was classified with an accuracy of 75% against *E. coli* and 87.5% against *S.* enteritidis (n=56). Finally, *E. coli* showed an accuracy of 67% (n=56) against *B. cereus* and 75% against *S.* enteritidis (n=56).
Significance: The results demonstrated the ability of glycan-coated magnetic nanoparticles to concentrate and extract pathogens and confirmed the applicability of near-infrared spectroscopy and machine learning classification algorithms towards pathogen identification. This technique does not require expensive recognition ligands and makes use of inexpensive spectrophotometer allowing affordable detection for mitigating food-related outbreaks.

P1-128 Performance Evaluation of a Loop-Mediated Isothermal Amplification (LAMP) – Bioluminescent Assay for Rapid Detection of *Salmonella* spp. from Boot Swabs in the Brazilian Poultry Industry

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Introduction: The industry's adoption of a farm-to-table quality control approach to food safety is growing. To ensure safety of the food even in the farm phase, several producers carry out the monitoring of *Salmonella* spp. on boot swabs. For Brazil, one of the most important poultry producers in the world, this type of analysis is frequent and very important. The increased demand for this type of testing requires methods that provide rapid and accurate detection of pathogens.
Purpose: To determine the specificity, sensitivity and accuracy of a Loop-Mediated Isothermal Amplification (LAMP)-bioluminescent assay for detection of *Salmonella* spp. in boot swabs compared to official method (BRAZIL, Portaria 126, 1995).
Methods: In this paired study, 40 samples of boot swabs from a local poultry farm were collected and used in this study. Twenty (20) naturally contaminated samples and 20 samples artificially contaminated with *Salmonella* Typhimurium (n=10 with 1-5 CFU/25g and n=10 with 5-10 CFU/25g) were analyzed using both methods. Samples were pre-enriched in BPW ISO at a 1:10 dilution and incubated at 37°C for 22-24h, and analyzed by the LAMP-bioluminescent assay and reference method. Sensitivity, specificity, accuracy and POD were determined.
Results: The LAMP-bioluminescent assay was able to detect *S.* Typhimurium in the boot swab samples. Compared to the traditional method, the overall sensitivity, specificity, and accuracy of the LAMP-bioluminescent assay was 99%, 99% and 99%, respectively. False positive and false negative results were not observed with the LAMP-bioluminescent assay. The POD analysis between the LAMP method and culture confirmation did not show any significant difference at a 95% confidence interval for all the matrices tested.
Significance: The alternative LAMP-bioluminescent molecular method enabled reliable and rapid detection of *Salmonella* spp. in boot swabs. The easy-to-use LAMP-bioluminescent method offers poultry producers a rapid method to screen primary production samples.

P1-129 Rapid Isolation and Detection of *Salmonella* Using Hollow Glass Microspheres Coated with Specific Antibodies

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is one of the most common microbes responsible for foodborne illnesses and mortalities. Considering the health risks associated with *Salmonella* in food, development of a simple bacterial isolation and detection system is vital for reducing foodborne infections. ELISA, PCR and other conventionally used methods are inaccessible to resource limited and remote areas. Self-floating hollow glass microspheres (HGMS) coated with layer-by-layer (LbL) nanostructured polymeric films and specific antibodies can be used for isolation and recovery of *Salmonella.*
Purpose: To develop a novel and cheap method for isolation and detection of *Salmonella* from food samples in resource limited areas.
Methods: LbL self-assembly technique was used to coat HGMS (size=20 µ, density=0.6 g/cc) with alternate positive and negatively charged polymeric layers. Polydiallyldimethylammonium chloride (PDDA) (2 mg/ml) was used as the positively charged polymer and biotinylated alginate (BALG) (2 mg/ml) was used as the negatively charged polymer with a washing step in between. Biotin-neutravidin conjugation was used to attach biotinylated anti-*Salmonella* antibodies (100 µg/ml) to these HGMS. Phosphate buffer saline (PBS) spiked with *Salmonella typhimurium* (ATCC 14028) (ST) (300 µl) was used to test the capture ability of coated HGMS. After 1h, HGMS were centrifuged and washed with PBS twice to separate them from uncaptured bacteria.
Results: Formation of polymeric films was confirmed in our previous studies by scanning electron microscopy (SEM) and profilometry. Thickness of the polymeric film was found to be approximately 50 nm. The presence of captured bacteria on washed HGMS was confirmed by SEM and by harvesting HGMS onto blood agar plates for 24h. HGMS covered with ST were seen under SEM and bacterial growth was observed on agar plates after 24h confirming presence of captured ST on HGMS surface.
Significance: This technique can be used to rapidly isolate and detect *Salmonella* in food samples in remote areas without any sophisticated equipment.

Poster

P1-130 Detection of *Listeria monocytogenes* in Environmental Sponge Swabs Using a Sponge Swab Rinsing Procedure as Compared to the FDA/Bam Standard Method

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Introduction: Environmental testing for pathogens is a vital component of a food safety program. Testing of multiple pathogens from a single swabbing site requires the collection of a sponge swab for each pathogen target. Each entire sponge is enriched for direct detection. A sponge rinsing procedure with incubation of the rinsate was investigated as an alternative to direct sponge enrichment to allow detection of multiple pathogens from one sponge.

Purpose: To assess whether detection of *L. monocytogenes* from cellulose and polyurethane sponge swab rinsates is equivalent to direct sponge enrichments.

Methods: Cellulose sponges with DE neutralizing broth and polyurethane sponges with HiCap™ broth were inoculated with *L. monocytogenes* at 0.25 CFU (n=10), 2.5 CFU (n=10), 25 CFU (n=5) and uninoculated (n=3); coinoculated with *Enterococcus faecium* (competitor) at 10x the pathogen inoculation level. Sponges were refrigerated 40h to simulate shipping conditions prior to enrichment. Using the FDA BAM reference procedure, Buffered Listeria Enrichment Broth (BLEB) was added to one set of reference sponges (direct enrichment) and incubated. Fifty ml Peptone water was added to another sponge set and homogenized for five or 30 seconds manually or by stomacher. Rinsates from each were transferred to BLEB and incubated. All incubated enrichments were streaked to MOX and chromogenic agars, and the plates examined for typical colonies.

Results: Unpaired statistical analysis compared rinsates to reference method sponges. Equivalence was evaluated using AOAC Equivalence Criteria. Equivalence is demonstrated when the observed dPOD and the 90% lower confidence limit for dPOD are greater than -0.20. At a 2.5 CFU contamination level, the results obtained from rinsed sponges were not equivalent to those obtained using the FDA BAM method regardless of sponge type, homogenization time or procedure.

Significance: Sponge rinsates result in a lower *Listeria* recovery rate and are not comparable to a standard direct enrichment (FDA BAM) for the detection of *Listeria* from environmental sponges.

P1-131 Rapid Detection of *Salmonella* spp. Using the Loop-Mediated Isothermal Amplification (LAMP) Assay – Bioluminescent in Primary Production Pre-Slaughtering and Sanitary Void Boot Swabs Collected from Brazilian Farms

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Introduction: Brazil is one of the world's largest exporters of poultry products. Sanitary measures and verification steps throughout poultry production are necessary, such as the analyses of broiler litter for monitoring and controlling *Salmonella* spp. These highly alkalinized samples are collected by dragging moistened boot swabs in the environment. Rapid and accurate *Salmonella* spp. detection in primary production samples is important to monitor effectiveness of control strategies and enable rapid corrective actions.

Purpose: This study aimed to compare the performance of LAMP-bioluminescent assay and reference confirmation (ISO 6579-1:2017) for *Salmonella* detection in primary production boot swabs.

Methods: Boot swabs samples (n=195) from three different Brazilian south region farms were collected, with two sets of samples from pre-slaughtering (each n=65) and one from sanitary void (n=65). Samples included broiler litter (e.g., soil, feathers, feces, wooden shavings, and lime powder) and sterile boot swabs moistened with BPW ISO. Fractional artificial contamination was used (five from each farm were blank and each strain (*Salmonella* Typhimurium ATCC 14028, *S.* Enteritidis wild strain and *S.* Pullorum ATCC 9120, all adapted to the matrix), was spiked at two levels (N1=0.1 and N2=1-2 CFU/test portion) with 20 samples each. Enrichment with BPW ISO was done at 1:10 for pre-slaughtering and 1:15 for sanitary void samples. Samples were incubated at 37°C for 20 hours, analyzed with LAMP-Bioluminescent assay and then confirmed by ISO 6579-1. Sensitivity, specificity, relative trueness (RT), relative limit of detection (RLOD), and acceptability limit (AL) were determined according to ISO 16140-2.

Results: Compared to reference procedure, the alternative LAMP-bioluminescent method sensitivity, specificity, RT, and RLOD were 100%, 99.2%, 99.5%, and 1.00, respectively. The alternative LAMP assay passed acceptance limit and can be considered fit for purpose.

Significance: The alternative LAMP method enabled reliable and rapid detection of *Salmonella* spp. in highly alkaline primary production pre-slaughtering and sanitary void boot swabs.

P1-132 Rapid Detection of *Salmonella enterica* in Dried Red Chile

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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.

Purpose: Four molecular techniques for detection of *Salmonella* in artificially contaminated red chile were evaluated against the FDA BAM cultural procedure for *Salmonella* detection.

Methods: Dried red chile powder was inoculated with a five-strain cocktail of lyophilized *Salmonella enterica* at a low level (~0.05 CFU/g) and a high level (~0.5 CFU/g). Uninoculated control samples were also examined. The samples were enriched in universal broth and buffered peptone water and incubated at 35˚C for 24h. The enriched samples were used for the ANSR test, the GENE UP test and for qPCR. Also, enriched samples were subcultured into Tetrathionate Brilliant Green and Rappaport–Vassiliadis broths and incubated for an additional 24h. Samples were prepared for qPCR, subcultured into M broth for the VIDAS assay and streaked onto selective agar plates (Hecktoen agar, Xylose Lysine Deoxycholate agar, Bismuth Sulfite agar and CHROMagar *Salmonella*). After incubation, typical *Salmonella* isolates were screened on Triple Sugar Iron and Lysine Iron Agar slants and confirmed with biochemical tests, serology, and by qPCR.

Results: When the molecular methods (ANSR, GENE-UP and qPCR) were performed directly from primary enrichments (TSB or BPW), the *Salmonella* detection rates were low when compared to the cultural method. After TT/RV enrichment, the BAM-qPCR, and VIDAS-SLM showed *Salmonella* detection rates that did not differ from the FDA-BAM cultural method ($\chi 2 < 3.84$).

Significance: These results show that a secondary enrichment improved *Salmonella* detection in red chile powder. Optimization of the ANSR, GENE-UP and qPCR procedures for use with chile powder without secondary enrichment is necessary before they can be used for this difficult matrix.

P1-133 *Salmonella* Quantification (SalQuant®) Utilizing the BAX® System for Pork Primary Production Spleen and Rope Samples

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Introduction: The spleen serves an important immune function in the body and ropes are often hung in hog pens as an enrichment device for the animals. These samples should be considered as they may provide information about *Salmonella* in lairage.

Poster

Purpose: This study was performed to develop and verify BAX® System SalQuant® as an efficient PCR quantification method of *Salmonella* in pig spleen and rope samples.

Methods: Spleen and rope samples were procured from a commercial pork producer, screened for *Salmonella*, and negative samples were inoculated with *Salmonella* Typhimurium ATCC 14028 at concentrations of 0.00 – 4.00 log10 CFU/g. Spleens were homogenized with buffered peptone water (100 mL) then 30 mL was combined with 30 mL media+1 mL/L of quant solution (QS). Ropes were enriched with 300 mL media QS. All samples incubated for 6, 8, or 10 h at 42°C and were quantified using real-time PCR for *Salmonella*. A 3×5 MPN was also conducted, and the two quantification procedures were compared using a paired t-test (GraphPad Prism 9 at P=0.05), while JMP® v.15 was used to calculate the R^2 and RMSE for *Salmonella* quantification curve development.

Results: When spleens were incubated at 42°C for 8h, the linear-fit model yielded an R^2 of 0.83 and Log RMSE of 0.62. When rope samples were incubated at 6 hours a linear-fit model with an R^2 of 0.81 and Log RMSE of 0.40 was the result. There was no statistical difference between MPN and *Salmonella* PCR quantification (P >0.05).

Significance: Results suggest that real-time PCR can accurately quantify *Salmonella* from pork spleen and rope samples after 8 hours and 6 hours of incubation, respectively. This provides the pork industry with another sample types that can be used to quantify *Salmonella* in the pork production environment.

P1-134 *Salmonella* Quantification (SalQuant®) Utilizing the BAX® System for Pork Primary Production Fecal Samples and Cecal Swabs

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Introduction: Pigs harbor *Salmonella* in their gastrointestinal tract, suggesting that feces and cecal swabs may be effective samples for *Salmonella* enumeration and understanding pathogen load.

***Purpose*:** This study was conducted to develop and verify BAX® System SalQuant® as a rapid PCR quantification tool for *Salmonella* in market hog fecal and cecal swab samples.

***Methods*:** Fresh feces (N=35; 10 g) were homogenized with 90 mL of media + quant solution (0.5 mL/L). Cecal swabs (N=35) were collected using a pre-moistened BPW swab and combined with 50 mL of media + quant solution (1 mL/L). Samples were screened for naturally-occurring *Salmonella* and 15 screened-negative samples were inoculated with *Salmonella* Typhimurium ATCC 14028 at 0.00 – 4.00 log10 CFU/g, while 12 screened-negative samples were inoculated at 5.00 – 8.00 log10 CFU/g (mL), for both sample types. Ten milliliters of each fecal slurry were combined with 10 mL of media + quant solution (0.5 mL/L), and samples incubated for 0 (high-level), 6, 8, or 10 h (low-level) at 42°C and quantified using the real-time PCR for *Salmonella*. A 3×9 MPN was conducted at each inoculation level and a paired t-test at P=0.05 was used to compare methodologies using GraphPad Prism 9, while JMP® v. 15 was used to calculate the R^2 and RMSE for curve development.

***Results*:** Fecal samples at 10 hours resulted in a linear-fit model with an R^2 of 0.84 and a Log RMSE of 0.61. High level fecal and cecal samples resulted in an R^2 of 0.88 and 0.75 and Log RMSE of 0.41 and 0.33, respectively. A statistical difference was not observed for the MPN and *Salmonella* PCR quantification methods (P>0.05).

***Significance*:** Real-time PCR can accurately quantify *Salmonella* in pig feces and cecal swabs with 10 and 0 h incubation, respectively. This allows the pork industry to quantify *Salmonella* efficiently in pre-harvest hogs.

P1-135 Application of a Novel Quantification Methodology for Enumeration of *Salmonella* in Beef Lymph Node Samples Collected during Harvest

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Introduction: Knowledge about *Salmonella* prevalence and concentration in beef Lymph Nodes (LN) is important for risk-based decision making on processing operations to determine effective mitigation strategies for decreasing the risk of *Salmonella* contamination in beef products.

Purpose: Determine the effectiveness of a novel RT-PCR-based quantification methodology to detect and enumerate *Salmonella* in beef lymph nodes collected in harvesting operations.

Methods: Five sample types (cecal content sponge, ileocecal, superficial cervical, popliteal, and subiliac LN) were obtained from 20 random carcasses of 16 pens out of 7 feedyards. *Salmonella* was enumerated and detected using BAX System SalQuant and BAX Real-time PCR *Salmonella* assays, respectively. Cecal content sponges and LN samples that were *Salmonella* prevalent but below the limits of quantification (1.69logCFU/swab; 1logCFU/LN) were assigned values of 0.84 logCFU/cecal content sample and 0.47 logCFU/LN sample, respectively. Samples below the limit of quantification were assigned values of 1.18 logCFU/cecal content sample and 0.84 logCFU/LN sample, respectively. Prevalence negative samples were assigned values of 0.00 logCFU/sample. A classification of the feedyards was made according to the quantification of Salmonella: <0.5 LogCFU/Sample = Lowlevels, 0.5-1.00 LogCFU/Sample = medium level and > 1 LogCFU/Sample = high levels. Kruskal–Wallis test was used. Pairwise comparisons were done using the Wilcox-test. Statistical significance was evaluated at 0.05 probability level.

Results: *High and medium Salmonella levels* were detected in samples collected from one feedyard each. Low *Salmonella* levels were presented in the remaining five feedyards. In samples collected from low levels feedyards, the highest enumeration levels of *Salmonella* were found in cecal sponge (0.202 logCFU/sample) and ileocecal LN (0.199 logCFU/sample). In high concentration feedyards, popliteal and subiliac LN presented the highest loads (>3.01 logCFU/sample), p-value=0.000021. In medium concentration feedyards, type of sample was no significant, p-value=1.00.

Significance: Identifying the risk level of sentinel nodes for potential *Salmonella* contamination, can guide processors to modify their processing operations to remove high risk LN to reduce the likelihood of *Salmonella* contamination in beef products.

P1-136 Development of an Automated Quantification Method for Enumeration of *Campylobacter* on Chicken Neck Skins

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Introduction: Chicken neck skins are known to have higher *Campylobacter spp*. levels compared to other skin locations. Rapid and accurate enumeration of *Campylobacter* on chicken neck skins would allow the poultry industry to make faster food safety decisions and protect public health.

Purpose: The purpose of this study was to develop an enumeration tool for *Campylobacter jejuni*, *coli*, and *lari* on chicken neck skins utilizing the BAX® System.

Methods:

Chicken neck skins (25 g) were screened negative using the BAX System and combined with 250 mL of BPW. A secondary enrichment (10 mL) was transferred and inoculated at 0.00-3.00 Log CFU/g with three biological replications for each inoculation level of *C. jejuni* (n=13), *coli* (n=13), and *lari* (n=13). Ten milliliters of pre-warmed (42˚C) 2X Bolton's Broth with 2X Supplement was added to each sample then incubated under microaerophilic conditions at 42˚C for 16 h prior to being analyzed by the BAX System in quadruplicate. Additionally, inoculated samples were spread plated in duplicate on TSA and MCCDA and incubated under microaerophilic conditions at 42˚C for 48 h before Log CFU/g was determined. Linear-fit equations of positive *Campylobacter spp*. CT values were created and compared to plate counts using 95% CI.

Results: At 16 h, CampyQuantä generated linear-fit curves for *C. jejuni*, *coli*, and *lari* with an R^2 of 0.60, 0.72, and 0.77 and Log RMSE of 0.28, 0.64, and 0.61, respectively. Plate counts yielded a 25, 75, and 25% on TSA and N/A, 100, and 0% comparison for 95% CI for *C. jejuni*, *coli*, and *lari*, respectively. However, CampyQuantä estimations were more accurate based on target inoculation levels.

Significance: Plate counts for *Campylobacter* are variable, however, providing the poultry industry with a rapid and reliable PCR-based enumeration tool for *Campylobacter* will allow for faster data-driven decisions resulting in a safer, more wholesome final product.

P1-137 *Salmonella* Quantification Utilizing Real-Time Polymerase Chain Reaction for the Development of Turkey Trailer Swab Samples

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* is a ubiquitous pathogen in the turkey industry. To effectively control *Salmonella* in turkey products, it is necessary to determine an efficient pre-harvest sampling method for quantification of *Salmonella.*

Purpose: This study was performed to develop and verify an effective real-time polymerase chain reaction (RT-PCR) method to enumerate *Salmonella* in turkey trailer drag swab samples.

Methods: Turkey trailer swab samples (N=35) were collected with pre-moistened MicroTally® swabs. A portion of each sample (1 mL) was incubated for 18-24 hours then pre-screened for *Salmonella* using the BAX® System PCR Assay for *Salmonella*. Screened negative samples were spiked with 0.00 to 4.00 Log CFU/mL of *Salmonella* Typhimurium 14028 with three samples per inoculation level, plus one uninoculated sample (n=16). Inoculated samples were hand massaged for one minute to create homogenates before 200 mL of BAX MP + 1 mL/1L of Quant Solution (QS) was added to each sample. Samples were incubated at 42 °C for 6, 8, and 10 hours then analyzed for *Salmonella* utilizing the BAX System at each time point with five lysates per sample. One MPN per inoculation level per sample type was performed for verification and tubes were incubated at 37 °C for 18-24 hours. JMP® v.15 was used to run linear regression on cycle threshold (CT) values to determine the R^2 and RMSE for quantification curve development.

Results: The most accurate linear-fit equation obtained was determined at 8-hours of incubation, with an R-squared of 0.63 and Log RMSE of 0.82. The MPN methodology continually underestimated, however, RT-PCR demonstrated more accurate estimations based on inoculation levels.

Significance: The development of a BAX SalQuant turkey trailer swabs provides the poultry industry with a helpful pre-harvest sample collection method to determine the incoming load of *Salmonella* in turkey production environments.

P1-138 Quantification of the Number of Viable but Non-Culturable *Campylobacter jejuni* by an Alternative Novel Technique Using Dielectrophoresis with Micro-Fluidic Device

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Introduction: One of the causes of foodborne illness by *Campylobacter jejuni* may be related to existence of viable but non-culturable (VBNC) cells due to morphological change from spiral to coccoid. Although the VBNC state cells can be quantified by some techniques, *C. jejuni* cells in VBNC state are difficult to isolate and collect as they are, without chemical modification.

Purpose: The aim of this study was to investigate the possibility to apply a novel technique using dielectrophoresis (DEP) with micro-fluidic device to quantify VBNC state *C. jejuni* cells without chemical labelling.

Methods: Pre-grown *C. jejuni* cells (10^8 CFU/ml) in Bolton broth were stored in phosphate buffer in a micro-aerobic condition at 4 and 37°C to investigate the occurrence of VBNC cells during storage. The culturable bacterial cells were enumerated by mCCDA culture media, and VBNC cells were quantified by the DEP procedure using the apparatus (ELESTA PixeeMo, AFI Corp., Japan) with 3000 to 7000 kHz frequencies to clarify the dielectric nature of *C. jejuni* cells. Furthermore, EMA-qPCR method was used as a reference procedure for detection of VBNC cells.

Results: Although culturable *C. jejuni* cells were rapidly decreased and not detected (<10^2 CFU/mL) after 24 h at 37°C storage, the live cell numbers detected by the DEP and EMA-qPCR methods showed different behaviors. The changes in the live cell numbers enumerated by the DEP method with 5000 kHz illustrated moderate decrease by 4 log CFU/mL in four days storage, which is similar to the results of the EMA-qPCR method. While the number of culturable *C. jejuni* cells gradually decreased for eight weeks, the live cell numbers did not change throughout the storage period.

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.

P1-139 Flow Cytometry in Probiotics: The Intersection of AFU and CFU

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Introduction: As probiotics become more prevalent in the market, there is increasing need for faster and more precise ways to measure viable cells in products. Flow cytometry (FC) is a promising candidate; however, disagreement between plate count (CFU/g) and FC (AFU/g) quantification has remained a sticking point for widespread industry adoption of this newer technology.

Purpose: The purpose of this study is to develop a robust analysis protocol (gating, instrument settings, dilutions) that allows for a more direct comparison of AFU and CFU values in powdered probiotic samples.

Methods: Ten mixed *Bacillus subtilis* and *Bacillus coagulans* samples were analyzed in triplicate using an Attune NxT Flow Cytometer and traditional plating methods. For FC and plating, powdered probiotic starter samples were rehydrated in a 1:1 0.9% saline:MRS mixture and then serially diluted in 0.9% saline to achieve appropriate levels for analysis. FC samples were stained using Syto9 and Sytox to differentiate live and dead cells. For plating, *B. subtilis* was plated on TSA and incubated at 35°C for 24 h before and *B. coagulans* was plated on GYE and incubated at 37°C for 48 h before counting.

Results: In eight out of 10 samples, no statistically significant difference ($P<0.05$ using paired t-test) was observed between the plating and FC counts, indicating the two methods generate similar results. Furthermore, all samples returned AFU and CFU values at or above the stated minimum CFU values.

Significance: This work identifies an analysis protocol that can be used consistently across multiple samples, which allows for deeper comparisons between CFU and AFU values in *B. subtilis* and *B. coagulans* probiotic powders.

P1-140 Genetic Engineering of a *Salmonella* Phage for Host Separation, Concentration, and Detection

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* contamination is a threat to public health, causing foodborne diseases and being responsible for many deaths around the world. Current methods to detect *Salmonella* needs a lab setting, are time consuming and can be inconclusive. Bacteriophages can be used as a diagnostic tool to bridge this gap.

Purpose: To design a fast and efficient way of genetically engineering *Salmonella* phages for their use as tools to capture, separate, concentrate, and detect their host in water and agricultural systems.

Poster

Methods: Competent *S. enterica* subsp. *enterica* ser. Typhimurium was co-transformed with a 2-plasmid system, pCRSIPR containing the guide RNA (gRNA) with the pCas12a that contain the gene for Cas12a and the S16 donor plasmid. The S16 bacteriophage (phage) was genetically engineered to have mSA (monomeric streptavidin) fused to Soc (nonessential capsid protein) to allow for conjugation to magnetic nanoparticles, and a luciferase (NanoLuc) gene to be expressed during host infection as a reporter enzyme. The efficiency of the engineering was determined following confirmation (sequencing and bioluminescence assay) of the genetic modification.

Results: Ten gRNA were tested, gRNA#7 showed a six-log drop in plaque forming unit compared to the control gRNA after screening against the S16 genome (*P*=0.007) suggesting efficient cutting of the phage genome. Following CRISPR-Cas genetic engineering using gRNA#7, 12 resulting plaques were selected for evaluation. All 12 plaques had the mSA insert while 6/12 were confirmed to have both the mSA and NanoLuc genes present. The six mSA-NanoLuc plaques showed solid bright blue luminesce following substrate addition during the bioluminescence assay.

Significance: An S16 bacteriophage was engineered to facilitate conjugation to magnetic particles and expression of a luciferase during infection of the host *Salmonella*. This has previously been demonstrated to allow the detection of <10 CFU per 100 ml of *E. coli* in water when engineering T4 phages.

P1-141 MALDI-TOF Mass Spectrometry with Machine Learning for High-Throughput Screening of Raw Milk for Evidence of Bacterial Contamination

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Introduction: Raw milk must be screened to prevent pathogenic microorganisms associated with mastitis from entering the food supply.

Purpose: This work evaluates whether MALDI-TOF-MS coupled to machine learning algorithms is a cost-effective method for high throughput screening of dairy milk samples.

Methods: The method involves first analyzing raw milk from animals by matrix-assisted laser desorption ionization mass spectrometry. Mass spectra for a training dataset of n=226 composite milk samples collected from a local dairy were acquired. Then, peaks from the mass spectra were imported into a machine learning model and this software application discovers non-obvious patterns present which coincide with the mastitis condition. Finally, a separate set of milk samples were analyzed (scoring set; n=100) to evaluate the diagnostic accuracy of the new model. Decision trees, random forest model, gradient boosted trees, and neural network models were investigated for optimal performance.

Results: Results suggest the gradient boosted decision tree model offers best performance, with simultaneous sensitivity and specificity >0.8 for mastitis diagnosis. The decision tree approach is conceptually simple and identifies candidate biomarker molecules indicative of the presence of mastitis.

Significance: The coupled MALDI TOF plus machine learning approach is effective for screening the presence of mastitis in dairy cows and is likely a viable option for diagnoses of a wide array of animal health concerns. Since the MALDI TOF method only requires a microliter of inexpensive reagents, the tool is amenable to high-throughput screening.

P1-142 Development of Magnetic Relaxation Switching-Based Assay for the Detection of Bacterial Pathogens in Food Matrices

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❖ ***Developing Scientist Entrant***

Introduction: Current standard methods of detecting foodborne contamination in industries such as plate culture and PCR have longer turnaround times and extensive sample processing steps and so, requires faster detection methods in the plant environment to improve food quality and safety.

Purpose: This study applied the concept of magnetic relaxation switching (MRS) mechanism to develop a detection assay that uses *Escherichia coli* as a model organism with minimal sample processing requirements.

Methods: Magnetic nanoparticles functionalized with amino groups of size around 50 nm were prepared through a novel one-pot solvothermal synthesis followed by surface modification with anti-*E. coli* goat polyclonal antibody (A-MNPs). *E. coli* cultures were prepared using TSB, diluted, and inoculated in PBS, and incubated with A-MNPs for 15 minutes before testing using 20 MHz low field NMR device. Carr-Purcell-Meiboom-Gill sequence was applied to detect the transverse (T_2) relaxation time of the water protons in the sample that changes with the behavior of the MNPs surrounding them. The changes in the T_2 times in samples with and without the bacteria were recorded within 10 minutes to confirm their detection.

Results: The addition of A-MNPs resulted in their spontaneous assembly on the *E. coli* surface through attachment to the antibody (microbial nanocoating). This led to a change in the T_2 relaxation times of the water protons around the nanoparticles which was measured using T_2-NMR. Results showed that the T_2 value for sample with MNP bound *E. coli* was found to be around 13 s, which was significantly higher than samples with MNPs that remained dispersed in the absence of *E. coli* (~5 s).

Significance: The developed MRS assay detected *E. coli* in the sample with minimal optical interferences since the detection is based on the magnetic properties of the attached MNPs, thereby, reducing the need for extensive sample preparation steps.

P1-143 Monomeric Streptavidin Phage Display Allows Efficient Immobilization of Bacteriophages on Magnetic Particles for the Capture, Separation, and Detection of Bacteria

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Introduction: Immobilization of bacteriophages onto solid supports such as magnetic particles has demonstrated ultralow detection limits as biosensors for the separation and detection of their host bacteria. While the potential impact of magnetized phages is high, the current methods of immobilization are either weak, costly, inefficient, or laborious making them less viable for commercialization. While streptavidin-biotin represents an ideal conjugation method, the functionalization of magnetic particles with streptavidin requires square meters of coverage and therefor is not amenable to a low-cost assay.

Purpose: Here, we genetically engineered bacteriophages to allow synthesis of a monomeric streptavidin on the phage capsid during infection of the bacterial host. The monomeric streptavidin was fused to a capsid protein (Hoc) to allow site-specific self-assembly of up to 155 fusion proteins per capsid.

Methods: Phages were genetically engineered using CRISPR/Cas9 to modify the capsid protein Hoc with an mSA fusion. Additionally, a gene for the luciferase NanoLuc was inserted into the phage genome resulting in expression during host infections. The modified phages were then conjugated to biotin-modified magnetic nanoparticles and used to capture *Escherichia coli* (ECOR13) from 100 ml water samples. Following capture, separation, concentration, and resuscitation, the samples were analyzed for active NanoLuc using a luminometer.

Results: The assay's signal increased proportionally with *E. coli* (ECOR13) concentration. The assay resulted in a 4.68 ± 1.82 luminescence signal:noise ratio for 10 ± 4 CFU/100 mL, and 23.85 ± 13.11 luminescence signal:noise ratio for 89 ± 19 CFU/100 ml. The limit of detection (LOD) for ECOR 13 in 100 ml of water using the mSA-modified phage assay was calculated to be ~5 CFU/100 ml.

Significance: This work highlights the creation of genetically modified bacteriophages with a novel capsid modification, expanding the potential for bacteriophage functionalized biotechnologies.

Poster

P1-144 Development of a Magnetic Nanoparticle Assisted Chemiluminescent Immunoassay for Detection of *Salmonella* Typhimurium in Foods

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Introduction: *Salmonella* is one of the main causes of food poisoning. *Salmonella* contamination may occur during production, processing, distribution, and marketing. Rapid and accurate identification of the contaminated food products is urgently needed to prevent foodborne illness outbreaks. The testing tools that can be deployable in processing and production settings would enable industry stakeholders to better provide a safe food supply.

Purpose: The purpose of this study was to develop a field-deployable chemiluminescent immunoassay in concert with the magnetic separation for rapid screening of *S.* Typhimurium in contaminated food products.

Methods: A workflow was designed for analyzing various types of food products (meats and leafy greens). The antibody-coupled immunomagnetic nanoparticles were applied to capture and concentrate *S.* Typhimurium in the samples through a magnetic column. Another antibody conjugated with an enzyme, horseradish peroxidase, was used to bind the captured *S.* Typhimurium and subsequently chemiluminescence reaction was catalyzed by the bacterium-bound peroxidase. The intensity of light was measured in Relative Luminescence Unit (RLU) using a portable luminometer.

Results: Food samples contaminated with *S.* Typhimurium at various levels (0 to 1.0×10^5 CFU/g) were evaluated and results were compared. The assay was highly sensitive and can be successfully performed within two hours in decentralized locations. The intensity of light (RLU) showed a log-linear correlation to the concentration of *S.* Typhimurium in the range of 5.6×10^1 to 8.1×10^4 CFU/g, with an R^2 value of 0.9965. The detection limits of *S.* Typhimurium in chicken meat and romaine lettuce were 3.2×10^2 and 8.5×10^1 CFU/g, respectively, without cross-reaction to other microbial strains.

Significance: The results suggested that it can be further developed into small portable measuring devices to facilitate preliminary screening tests. With such devices, the specific contamination sources can be traced along the processing and distribution lines in a timely and economic manner.

P1-145 Development of a Microfluidic "Lab-on-a-Chip" Device to Detect Mycotoxin Zearalenone in Foods and Feeds

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❖ ***Developing Scientist Entrant***

Introduction: The prevalent occurrence of mycotoxin contamination in various foods and feeds poses health risks to humans and livestock, while current standard analytical methods are high-cost and labour-intensive with relatively low capacity.

Purpose: This study aims to develop a portable, automated, low-cost, and rapid microfluidic "lab-on-a-chip" device to detect mycotoxin zearalenone in foods and feeds.

Methods: The microfluidic analytical device was fabricated via photolithography, involving the design of photo masks with desired patterns, fabrication of wafer master with SU8 photoresist, and production followed by assembly of polydimethylsiloxane parts into lab-on-a-chip. Quantum-dots-embedded molecularly imprinted polymer (QD-MIP) was prepared with a synthesized dummy template via the sol-gel method. Food samples (e.g., wheat) were homogenized with a blender and extracted with 90% acetonitrile, followed by sedimentation for 1 min. The liquid extract was injected into the device with a micro pump and then enriched via evaporation assisted by the microfluidic annular flow. The extract was then mixed with QD-MIP flow, resulting in fluorescence signal quenching that quantifies mycotoxin concentration.

Results: The successful synthesis of the dummy template, CDHB, was confirmed via mass spectrometry (m/z = 319.19). In the fluorescence quenching test, the synthesized QD-MIP achieved a limit of quantification (LOQ) at 1 ppm zearalenone. The extract-enrichment module of the device achieved a tenfold concentration upon detection using QD-MIP, bringing the LOQ down to 0.1 ppm for grain samples. Each sample took ~30 min for the entire test from sample preparation to data analysis.

Significance: The developed microfluidic device would be a useful and portable tool for rapidly detecting mycotoxin levels in foods and feeds. This project benefits the monitoring of mycotoxin contamination in the agri-food sector and contributes to food safety and security.

P1-146 Title: Detection of *Campylobacter jejuni U*sing a Hybrid Paper/Polymer-Based Microfluidic Device Based on the Recombinase Polymerase Amplification and Lateral Flow Assay

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Introduction: *Campylobacter jejuni* is recognized as the leading cause of foodborne disease in poultry products globally. Traditional methods involving tedious microbiological culture isolation and PCR-detection cannot achieve the use either on site or in resource-limited settings. Recombinase polymerase amplification (RPA) is a robust isothermal amplification method that is capable of rapidly amplifying nucleic acids.

Purpose: A hybrid paper/polydimethylsiloxane (PDMS)-based microfluidic device integrating paper-based DNA extraction, RPA reaction and lateral flow assay (LFA) was developed to achieve rapid detection of *C. jejuni.*

Methods: For the specificity test, *six C. jejuni* wild isolates, nine other *Campylobacter* subspecies strains and 11 non-*Campylobacter* strains were investigated. Cellulose paper dipstick and RPA reaction were used to extract bacterial nucleic acids and amplify DNA extraction at 40°C for 20 min. When the sample containing the targeted analyte moved through the lateral flow strip, either a positive (two lines) or negative result (one line) could be evaluated by naked eyes.

Results: RPA reaction demonstrated 100% specificity to *C. jejuni*. The limit of detection on the device for pure bacterial culture was 460 CFU/ml. Besides, the device could detect *C. jejuni* spiked from 10 to 10^2 CFU/ml on chicken breast after enrichment for 5 to 10 h. For spiking *C. jejuni* $>10^3$ CFU/mL, the device managed to confirm positive results immediately. RPA reagents and primers could be lyophilized and stored on paper platform for 25 days.

Significance: We proposed a rapid and reliable approach for the detection of *C. jejuni* at low cost by using a microfluidic device based on RPA and LFA.

P1-147 Development of a Real-Time Biosensor to Detect Foodborne Pathogens in Leafy Greens Production Environments

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Introduction: A real-time biosensor, made of a phospholipid component of lung surfactant (biofilm), that moves in response to microorganisms was developed.

Purpose: To refine the prototype with upgraded optics to increase sensitivity and evaluate detection of foodborne pathogens and background microflora for generating preliminary data, to feed artificial intelligence (AI) computer algorithms for sensor curve pattern matching.

Methods: The biosensor was upgraded with new optic filters, to capture wavelengths ranging 617.5 nm to 632.5 nm, and replace the original filters ranging from 300 to 1,200 nm. This upgraded biosensor was tested with various aerosolized microbial inoculum levels in deionized water. Three trial measurements at a response rate of 0.015 seconds were conducted.

Poster

Results: Interaction of the aerosolized bacteria with the biofilm in the biosensor created changes in biofilm reflectance that was captured by a spectrometer as waves of reflected light intensity and wavelengths off the biofilm. This movement was plotted as mm difference in light intensity vs. wavelength (nm). The biosensor generated separate and different curves for *Salmonella*, *E. coli* O157:H7, *E. coli* K12, *Listeria monocytogenes*, and background microflora including *Pseudomonas fluorescens*, *Lactobacillus*, and yeast at inoculum levels of 10 cells/ml, 100 cells/ml, 1,000 cells/ml, 10,000 cells/ml, 100,000 cells/ml, 1,000,000 cells/ml, 10,000,000 cells/ml. Better resolution was demonstrated with a range of 2.0 to 13.0 mm light intensity vs. 623.7 to 627.7 nm wavelength with upgraded optics compared to previous ranges of 0.93 to 3.33 mm light intensity vs. 622 to 631 nm wavelength. These data sets confirmed that the 15 nm bandwidth allowed higher sensitivity and resolution between various organisms and inoculum levels, appropriate for AI techniques.

Significance: Continuous quantitative readings of foodborne pathogens at various locations in processing houses would allow rapid screening and help prevent foodborne outbreaks. This is critical to maintaining the integrity and sustainability of the US leafy green production systems.

P1-148 Method Comparison and Interlaboratory Study for the ISO 16140-6:2019 Validation of Check and Trace *Salmonella* 2.0, for the Confirmation and Typing of *Salmonella* spp.

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Introduction: Standard protocols for detection of *Salmonella* are time-consuming taking several days to generate a final positive or negative test result (ISO 6579-1 and ISO 6579-3). This study reports a quick and simple multiplex real-time PCR-test to confirm and type *Salmonella* spp. within 2.5 h from colony.

Purpose: Method comparison and interlaboratory study for the ISO 16140-6:2019 validation of Check & Trace Salmonella 2.0, for the confirmation and typing of *Salmonella* spp.

Methods: ISO 16140-6 was used as reference. For the method comparison study, strains were inoculated on Nutrient Agar (NA) and Xylose Lysine Deoxycholate agar (XLD) and tested on both the Biorad CFX96 and Biorad CFX Opus real-time machine. The amount of tested samples was according to ISO 16140-6 for both the inclusivity and exclusivity study for confirmation of *Salmonella* spp. and serotyping of 59 serovars (5 strains per serovar). For inclusivity, 313 *Salmonella* spp. strains were used and for exclusivity 175 non-target strains. For the interlaboratory study a total of 30 strains were tested by 15 operators in 13 different laboratories.

Results: For confirmation no deviating results were found. For typing, a few deviations were found, but were all within the acceptability limits described in ISO 16140-6 (AL: deviations ≤ 3 for both inclusivity and exclusivity). For the interlaboratory study a few deviations were found within the acceptability limits (AL: deviations ≤ 3 for both inclusivity and exclusivity). The presented test is a validated alternative method for ISO 6579-1 and ISO 6579-3 for the confirmation and typing of *Salmonella* spp.

Significance: Food producers or laboratories testing for *Salmonella* are able to use the test as a validated alternative method for ISO 6579-1 and ISO 6579-3 for the confirmation and typing of *Salmonella* spp.

P1-149 Fluorescent Detection of *Salmonella* in Food Systems Using a Graphene-Oxide-CRISPR (GO-CRISPR) System

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❖ ***Developing Scientist Entrant***

Introduction: Each year, foodborne pathogens cause over 48 million illnesses and cost over $15 billion in U.S. *Salmonella* is the most common cause of foodborne disease and frequently eludes detection along the food supply chain. This often sparks outbreaks severely harming both the public and the food industry. Traditional methods for *Salmonella* detection rely on colony counting or polymerase chain reaction (PCR), both of which are time-consuming and difficult to carry out on-site. While several point-of-care devices have been developed, they often lack sensitivity and affordability.

Purpose: Henceforth, we have developed a graphene-oxide-CRISPR (GO-CRISPR) assay for sensitive on-site detection of *Salmonella*.

Methods: Our strategy utilizes a CRISPR-Cas12a system for target-induced *trans*-cleavage of a single-stranded DNA (ssDNA) FAM-labeled probe. The *trans*-cleavage reaction separates the FAM probe from a poly(A) tail. In undegraded probes, the poly(A) tail will attach to graphene oxide (GO) for fluorescent quenching. However, probes degraded by the target-specific *trans*-cleavage will be separated from the poly(A) tail and remain unquenched. This produces a fluorescent signal indicating the presence of *Salmonella*.

Results: Our GO-CRISPR system can successfully detect 10 nM of *Salmonella* DNA ($p < 0.05$, Student's unpaired *t*-test), which is within a sensitive limit when paired with recombinase polymerase amplification (RPA) for isothermal amplification. The entire system can be carried out on-site to provide specific and sensitive *Salmonella* detection within 90 minutes.

Significance: Our assay provides a tool to vastly expand *Salmonella* detection within food systems and prevent countless outbreaks of foodborne disease. Additionally, due to its effectiveness and simplicity, our GO-CRISPR method can be expanded for the detection of similar dangerous foodborne pathogens within our food systems.

P1-150 ChapterDx MLSTnext NGS Technology for High-Resolution Genotyping/Serotyping of *Legionella*, *Listeria* and *Salmonella*, Shigatoxigenic *E. coli* (STEC)

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Introduction: Although Whole Genome Sequencing (WGS) generates complete genomic profile of food pathogens, it is a laborious, time-consuming, and expensive method that requires pure isolates, making it unsuitable for samples with complex background and thus, limiting its widespread applications by food industry. On the other hand, conventional MLST approaches do not provide sufficient SNP information to track-and-trace sources of contamination.

Purpose: To develop a high-resolution and cost-efficient NGS assay with the capabilities of 1) genotyping/serotyping, 2) subtyping, 3) detection of multiple genotypes/serotypes in the same sample, and 4) tracking-and-tracing the contamination source from sample to sequencing.

Methods: ChapterDx MLSTnext NGS assays amplify and sequence between 50 and 100 polymorphic loci, evenly spanning the target genome with the proprietary X1 technology, which can efficiently and effectively amplify up to 100 targets in a single-tube and single-step PCR reaction. In this study, 70 previously culture confirmed samples of *Legionella*, *Listeria*, *Salmonella* and STEC isolates (including ATCC isolates) were evaluated by this NGS technology.

Results: The four NGS assays targeting *Legionella*, *Listeria*, *Salmonella* and STEC generated high-resolution sequence results that were able to genotype/serotype each relevant isolate. Moreover, each assay could detect co-presence of multiple isolates with different genotypes/serotypes in the same sample. The loci profile for each sample also allows to track-and-trace the source of contamination.

Significance: The food industry is in need of a simple, effective, widely applicable and cost-efficient method to not only detect, but also determine the genotypes/serotypes and potential sources of multiple organisms in food and environment. These NGS assays generate high resolution sequence data for genotyping/serotyping, subtyping, and detecting co-presence of multiple genotypes/serotypes, and tracking the source of contamination. This NGS technology is one of the simplest NGS assays. It takes less than 24 hours to analyze up to 1000 samples in a single sequencing run.

P1-151 Subtyping of *Listeria innocua* Using a Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) for Proactive Source Tracking and Mitigation

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Introduction: *Listeria innocua* is an important hygiene indicator in the food industry. Subtyping *L. innocua* isolates helps track their potential sources, develop targeted control practices and effectively minimize *Listeria* transmission.

Purpose: To establish a rapid and reliable MLVA method for subtyping *L. innocua* isolates.

Methods: DNA was amplified in a single PCR reaction using fluorescently labeled forward primers based on six specific loci. The PCR fragments were separated using an ABI 3730XL Genetic Analyzer. The fragments were analyzed using GeneMapper™ and Geneious software programs.

Results: Twelve primer sets were selected or designed based on published loci or sequences and evaluated using 62 validation strains isolated from various food and environmental sources. Six of the primer pairs, confirmed *in silico* as specific to *L. innocua,* were selected for inclusion in the MLVA assay based on their amplification and discrimination capacity. The optimized assay was able to amplify all six loci in a single reaction and detected all *L. innocua* isolates (n=50), but none of *L. welshimeri* and *L. monocytogenes* isolates (n = 12). The analysis resulted in 33 MLVA types from the 50 *L. innocua* isolates of various sources with known related isolates grouping together and unrelated isolates separated in different groups. The Hunter-Gaston diversity indices ranged from 0.53 to 0.86 for individual loci while the diversity index was 0.96 for 6 loci combined, which is higher than the acceptable value of >0.9. A direct conversion approach from PCR fragment size to VNTR copy number was implemented to eliminate potential data interpretation variations. The method was reproducible and repeatable and successfully applied in analysis of over 110 isolates from food production facilities.

Significance: The MLVA method can be used to generate timely and actionable results for proactive and targeted monitoring/prevention practices to minimize listeria transmission and foodborne listeriosis.

P1-152 Evaluation of a Metabarcoding Method Against Standard Methods for the Detection of Common Foodborne Pathogens in Foods

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Introduction: Current methods are limited to detecting one or a few foodborne pathogens at a time. Simultaneous detection of common pathogens in one test would be extremely beneficial.

Purpose: To evaluate a culture-independent metabarcoding method against standard culture methods for testing foodborne pathogens in foods.

Methods: DNA was extracted from enrichments of 641 uninoculated and 185 artificially inoculated (at a fractional-positive level) foods and amplified by PCR based on the 16S and 23S rRNA genes. Amplicons were sequenced using an Illumina MiSeq sequencer. Sequences were analyzed using BaseSpace or Geneious software to identify bacterial species based on Greengenes (16S) and custom (23S) databases.

Results: From 205 uninoculated raw meat samples, the metabarcoding method detected 92/96 (95.8%), 31/32 (96.9%) and 37/47 (78.7%) of the culture positives, and eight, 20 and 43 additional positives for *Campylobacter jejuni/lari*, *Listeria monocytogenes* and *Salmonella enterica*, respectively. For 160 RTE meat, 120 dairy and 83 fruit/vegetable samples, there were no culture positives for the above pathogens while the metabarcoding method detected eight and 129 positives for *L. monocytogenes* and *Salmonella*, respectively. Among 73 uninoculated seafood samples, the metabarcoding method detected 62/68 (91.2%) *Vibrio* culture positives, predominantly *V. alginolyticus*, *V. parahaemolyticus* and *V. cholerae*, and two additional positives. For 94 raw and RTE meat, dairy and fruit/vegetable samples spiked with *Salmonella,* the metabarcoding method detected 40/44 (90.9%) of the culture positives and four additional positives. Among 91 samples of RTE meat, dairy, seafood and fruit/vegetables spiked with *L. monocytogenes*, the metabarcoding method detected 47/48 (97.9%) of the culture-positives with 16 additional positives. The discrepancy between the methods may be resolved by improved sample preparation and data analysis procedures and will be further investigated.

Significance: The results showed the potential of the metabarcoding method for detecting multiple foodborne pathogens in a single test and allowed identification of gaps for future improvements.

P1-153 Validation of Suitable Genetic Analysis Method for *E. coli* O157:H7 Belonging to Atypical Enteropathogenic *E. coli*

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Introduction: Regulation of *E. coli* O157:H7 is being strengthened, and methodology to confirm atypical cases becomes important.

Purpose: This study aims to provide an accurate detection method for *E. coli* O157:H7 belonging to atypical enteropathogenic *E. coli* not containing the *stx* gene.

Methods: *E. coli* O157:H7 strains isolated from intentionally contaminated samples for the proficiency test were used. The basic screening for *stx* and *eae* genes was performed with two methods, the PCR assay and 3M™ Molecular Detection System. Whole-genome sequencing (WGS) was conducted on an Illumina Miseq system and Oxford Nanopore MinION system through a TruSeq Nano DNA prep kit and Ligation Sequencing kit to confirm serotype and toxin genes. The final genome sequencing data was confirmed in concordance with the NCBI and Uniport databases.

Results: In the results of the PCR assay and 3M™ Molecular Detection System, the *stx* gene was not detected and only the *eae* gene was detected. The genome sequence of this strain was 5,441,747 bp in length, and the genome assemblies were obtained 2 contigs. As a result of WGS data analysis, it was identified as O157:H7 serotype and did not have the *stx* gene, whereas the *eae* gene was present. Since it could not be detected by the official method of Ministry of Food and Drug Safety (MFDS) in South Korea for enterohemorrhagic *E. coli* test method using *stx*1 *and stx*2 gene detection, additional analysis of the *eae* gene should be required to find a more accurate confirmation method.

Significance: For the accurate analysis of *E. coli* O157:H7, the official method of MFDS in South Korea should be improved based on the method for identifying both *stx* and *eae* genes. This study can be helpful in introducing an improved test method.

Poster

P1-154 Identification of *Listeria monocytogenes* through Oxford Nanopore-Based Whole Genome Sequencing

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Introduction: Pathogen contamination including *Listeria monocytogenes* in processed food products is a major challenge for food plants globally. Currently within the food industry, for any *Listeria* spp. and *Listeria monocytogenes* positive results generated by conventional agar detection methods (e.g., Agar Listeria according to Ottaviani and Agosti -ALOA) or rapid detection methods (e.g., VIDAS), the true identity of the micro-organisms must be confirmed by a series of biochemical confirmation tests taking more than two – four days. These conventional biochemical methods are time consuming, labor intensive, imprecise, and do not account for the genetic diversity of *Listeria monocytogenes*, which is critical for identifying the true genesis of the contamination.

Purpose: To overcome such limitations, we investigated a rapid molecular-based Listeria monocytogenes identification and genome comparison method: Oxford Nanopore Technologies (ONT)-based whole genome sequencing (WGS) method.

Methods: We sequenced the whole genomes of 21 *Listeria* spp. isolates including 3 *Listeria monocytogenes* with the ONT sequencer GridION for 24 hours, with 7 isolates multiplexed in each flow cell. Taking results from Illumina data as benchmark, we assessed the accuracy and efficiency of using ONT data to identify the species of the isolates tested. ANIb pipeline and *Listeria* Sequence Typing scheme was used for both ONT and Illumina data.

Results: We found that, ONT WGS data could accurately identify all the *Listeria* isolates representing 17 different *Listeria* species. The ANIb similarity of these isolates using ONT WGS data were comparable with using Illumina data, and ST clustering were comparable between using ONT and Illumina WGS data as well.

Significance: We demonstrated that ONT-based WGS method can be used as a rapid confirmation method for *Listeria* spp. for the food industry, and the information of the genetic diversity of *Listeria monocytogenes* from the ONT WGS data can be further used for identifying the true genesis of the contamination.

P1-155 Development of Foodborne Bacteria Detection Method Using Next-Generation Sequencing

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Introduction: New and mutated foodborne bacteria have been emerging due to diversified foods and climate change. It is difficult to block spreading of foodborne bacteria in time due to the low rate of identification from the causative sources.

Purpose*:* When a food poisoning occur, it is difficult to identify the causing agent. So it is necessary to study the detection and analysis based on genomics to accurately identify the source.

Methods: We reviewed domestic and foreign literature researches. We obtained species-specific genes and select them as candidates NGS gene panel to detect foodborne bacteria. For the production of the NGS panel, the genome information including all of the pathogenic genes was selected as the standard genome.

For the verification of the developed panel, specificity was confirmed by comparing the data obtained from in silico method with the results of the NGS panel.

And, We checked the sensitivity of the panel for each foodborne pathogen.

Results: A gene panel was created by selecting 692 targets, 146 genes of 17 foodborne bacteria.

The panel verification results showed 100% agreement with the in silico results and bacteria strain NGS results.

The detection limits of the foodborne pathogen NGS gene panel were found to be 0.3 to 3 pg at strain-specific DNA levels.

Significance: The genome data of foodborne bacteria can be used for future foodborne outbreak investigations and comparative genome analysis of foodborne bacteria.

P1-156 Comparison of Target Amplicon Sequencing Using the Miseq and Gridion Next Generation Sequencing Platforms for Detection of Foodborne Pathogens

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Introduction: The use of Whole Genome Sequencing (WGS) for pathogen detection has increased the accuracy and reduced the time for traceback and source attribution in foodborne outbreaks. However, low level contaminants may go undetected due to challenges in isolating them from food matrices or due to a high background of other microbial flora. We have previously shown using the Illumina platform that a custom targeted amplicon sequencing (TAS) panel offers increased sensitivity and specificity for pathogen detection in "spike-in" experiments. The turnaround time from isolation of DNA to obtaining data is two days. Nanopore GridION platform offers a sequencing approach that enables direct near real-time sequencing thus saving time to get results.

Purpose: The objective of this work is to provide a rapid and sensitive method using targeted detection of low-level pathogen contamination in complex samples and thereby positively impacting the use of metagenomics as a rapid screening method for pathogen detection.

Methods: DNA reference material from 10 strains (NIST RM 8376) was used to compare the limit of pathogen detection using the TAS panel. Three dilutions of the reference material were used to generate amplicons. Respective library preparation methods were employed for the MiSeq and GridION sequencers. GalaxyTrakr and BLAST matching of the amplicons was used for data analysis.

Results: Preliminary results show that both sequencing platforms using their respective analysis pipelines detect specific pathogens and their associated virulence genes at similar levels. However, the GridION offers a near real-time sequencing advantage resulting in obtaining data in less than an hour.

Significance: This study shows proof of concept that targeted sequencing to detect pathogens yields similar results with either GridION or MiSeq but GridION is faster as it can provide data in real time.

P1-157 High-Throughput Automated DNA Extraction: Is It Possible to Obtain High-Quality Shiga Toxin-Producing *Escherichia coli* DNA from Different Environmental Matrices?

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Introduction: Leafy greens-associated Shiga toxin-producing *Escherichia coli* (STEC) outbreak responses have involved the collection and evaluation of water, sediment, soil, and air samples in addition to produce. Such large-scale sampling efforts necessitate the assessment of automated high-throughput processes that yield DNA compatible with PCR.

Purpose: Evaluate the recovery and quality of STEC DNA from different environmental matrices using the Maxwell® RCS 48 Instrument with two different protocols as compared to manual DNA preparation.

Poster

Methods: From 77 enriched samples, DNA was extracted from aliquots using the PureFood Pathogen (PFP) and Fecal Microbiome DNA (FM) kits and manually processed by either a wash-spin-boil or Qiagen DNEasy step. DNA products were analyzed for STEC genes using PCR according to FDA BAM Chapter 4A.

Results: No statistical difference between the manual and automated DNA preparation methods was observed for air, water, and soil (including drag swab) samples when PCR Ct values were < 30.0 ($p < 0.05$). Notably, of the 39 soil samples manually extracted without STEC targets detected, DNA from the PFP and FM kits yielded positive results for 39 (Ct values > 33.4) and 13 samples (Ct values > 37.4), respectively. Similarly, 10 air samples without positive STEC detection from DNA manually prepared produced values when either PFP (n = 10, Ct > 33.7) or FM (n = 4, Ct > 38.3) extracted. Although only eight sediment samples were tested, all but one yielded PCR inhibition of the amplification control in DNA extracted using the PFP method but none in samples extracted using FM or manual methods.

Significance: In air, water, and soil samples with low-level STEC contamination as judged by PCR Ct values, the automated PFP and FM methods yielded detectable DNA when compared to manual procedures. However, the processing of sediment samples illustrated that performance of methods differed in their ability to remove PCR inhibitors.

P1-158 Detection of *Salmonella enterica* Plus 13 Serotypes of Concern in Poultry Rinse Matrix by Sero^{X}, a DNA Microarray-Based Detection System

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Introduction: The rapid and timely identification of *Salmonella enterica* serovars that are relevant to human health within poultry production chains is critical to reducing consumer food risks.

Purpose: This study sought to develop and evaluate a rapid DNA microarray screening assay that can detect *S. enterica* spp. as well as 13 sero-specific genes in a poultry rinse matrix, as a single multiplex test. Serotypes targeted include: Enteritidis, Typhimurium, Newport, Javiana, I 4,[5],12:i:-, Heidelberg, Muenchen, Saintpaul, Montevideo, Infantis, Braenderup, Oranienberg, and Thompson.

Methods: Sero-specific genes were identified from literature and gene specific PCR primers and probes were designed so that the entire set of gene targets could be multiplexed as a single PCR reaction for direct hybridization to a microarray printed in a 96-well format. Experimental specificity testing included screening a gDNA collection of 133 serotyped *S. enterica* strains. To investigate sensitivity, whole bird rinse samples were prepared in accordance with USDA MLG 4.10, artificially inoculated with *S. enterica* Typhimurium at target concentrations (0, 3, and 6 CFU/mL) and incubated for 20 hours at 37°C (n=30). DNA extraction was performed with a commercial magbead extraction kit.

Results: For specificity, the system accurately identified all 133 strains as *S. enterica* spp. and correctly characterized the serotypes of 122/133 of strains tested. After 20-hour enrichment with buffered peptone water (BPW) poultry rinse, the Salmonella enterica spp. and Typhimurium target were detected in 100% of the samples inoculated at the 6 CFU/mL level (n=10). The overall analysis time was between 24-26 h, including the initial 20hrs of enrichment culture.

Significance: This [multiplexPCR + DNA microarray] assay allows for rapid, inexpensive, high throughput molecular detection of *S. enterica* spp. and 13 prevalent sero-specific genes. Further refinement of the PCR reaction, thermocycling conditions, and additional gene markers may improve the specificity and sensitivity.

P1-159 Evolution of Hybridization Sequencing to Improve Detection of *Salmonella* in Environmental and Outbreak Samples

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Introduction: Culture-dependent metagenomic methods for *Salmonella* detection in complex matrices can have limited sensitivity due to low relative abundance of *Salmonella.*

Purpose: First and second-generation baits designs are compared to identify improvements in serovar detection between iterations and un-targeted metagenomic sequencing.

Methods: First-generation (Gen1) *Salmonella enterica* subsp. *enterica* specific RNA baits (~5000 80nt/probe) were designed from the SeqSero2 database of 373 O- and H-antigen sequences. Second-generation (Gen2) baits (~3000 120nt/probe) added four virulence genes (*inv*A, *mgtC*, *sopB*/*sigD*, and *spiC*/*ssaB*) to the Gen1 design. Benchmark samples were prepared with 1%, 10%, or 50% of serovars Mississippi ($10.3x10^{8}$CFU/mL) or Midway ($5.3x10^{8}$CFU/mL) with and without the competitor *Citrobacter braaki* ($5.5x10^{8}$CFU/mL) spiked into a manure background. Hybridization reactions were performed on Gen1 and Gen2 baits. Peach leaves (n=3) and fruits (n=2) implicated in an outbreak were spiked with a control strain of *S*. Gaminara. After 24-h pre-enrichment followed by 24-h selective enrichment, samples were sequenced with and without hybridization to Gen1 baits. Data were analyzed with SeqSero2 and the custom bettercallsal pipeline (https://github.com/CFSAN-Biostatistics/bettercallsal).

Results: Hybridization sequencing improved serovar detection in outbreak and benchmark samples over un-targeted metagenomic sequencing. For benchmark samples hybridized to Gen1(n=10) or Gen2 (n=9) baits, bettercallsal correctly identified 90 and 100% of spiked serovars, respectively, regardless presence of competitors or spiking level. SeqSero2 incorrectly predicted serovars in multi-serovar and competitor-positive samples with (n=3). In un-hybridized benchmark samples, bettercallsal and SeqSero2 both identified 9/12 samples correctly, but Seqsero2 mis-called one sample and failed to predict mixed-serovars (n=2). Serovars were assigned with bettercallsal for all peach outbreak samples (n=15) hybridized to baits but only 40% were predicted by SeqSero2. For un-hybridized sequences, bettercallsal identified *Salmonella* in 40% of samples while SeqSero2 predicted serovars in 53% of samples.

Significance: Hybridization sequencing, in combination with bettercallsal, greatly improves *Salmonella* detection and serovar calling in environmental and outbreak samples.

P1-160 Evaluation of the 3M™ Molecular Detection Assay 2 for the Detection of *Salmonella* in Low-Moisture Foods

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Introduction: The 3M™ *Salmonella* Molecular Detection Assay 2 employs loop-mediated isothermal amplification to rapidly amplify nucleic acid sequences with high specificity and sensitivity, utilizing bioluminescence to detect the amplification of the target from food and environmental samples.

Purpose: The performance of this alternative method was compared to the Canadian culture-based reference method MFHPB-20 and evaluated according to Health Canada, Microbiological Methods Committee (MMC) guidelines for the relative validation of qualitative microbiological methods as a scope extension for low moisture foods.

Poster

Methods: Unpaired samples inoculated with *Salmonella* spp. previously isolated from low moisture foods were analyzed by the alternative and reference methods. Chocolate based products (including Low moisture, Bakery and Dry products), powdered dairy products and whole nuts were inoculated at three levels: 20 samples at a level (L_1) likely to give fractional positive results (25-75%), 20 samples at a high level (L_2) at approximately 10 times L_1, and 5 un-inoculated samples. Alternative samples were enriched in BPW broth for Whole Nuts and Dry Dairy; and Skim Milk Medium with 0.002% brilliant green dye for Chocolate-based products, and tested at 18, 20 and 24h of incubation at 37 ± 1°C. All analytical outcomes were culture confirmed by the reference method.

Results: Collectively, from the analysis of 540 unpaired samples, a probability of detection (POD) statistical model determined the alternative method met the criteria outlined by the MMC obtaining a relative sensitivity of 100%, relative specificity of 99%, a false positive rate of 1%, a false negative rate of 0% and test efficacy of 99.6%.

Significance: The Alternative method is suitable for detecting *Salmonella* spp. in a variety of low moisture foods, thereby significantly reducing reporting times over the reference method.

P1-161 Cereusid a User-Friendly Tool to Identify Isolates and Hazard within *Bacillus cereus* Group

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Introduction: Presumptive *Bacillus cereus*, also known as *Bacillus cereus sensu lato* or *B. cereus* Group, includes closely related Gram-positive, spore-forming and aerobic bacilli, that are widely distributed in the environment and food matrices. At present, more than 20 species have been described in this Group. Besides characteristic colonies on Mossel agar, these species may exhibit highly divergent properties and their distinction remains challenging.

Purpose: To develop a user-friendly tool ("cereusID") to clarify phylogenetic position of presumptive *B. cereus* and ease the identification of associated hazards.

Methods: A user-friendly web interface was developped specifically for *B. cereus* Group strains, integrating (i) a comprehensive database of gene sequences from representative genomes, (ii) a specific program allowing sequence comparisons, and (iii) predicted strain characteristics. Particular attention was paid to include all the most exhaustive phylogenetic and phenotypic diversity yet encountered within the *B. cereus* Group.

Results: CereusID user-friendly tool will be freely available online soon to ease strain identification. Results are given in the form of (i) sequence query, (ii) strain affiliation with a specific phylogenetic group or sub-group at a % identity level, and its phylogenetic position on a all-encompassing Tree including all known species, (iii) specific characteristics enabling hazard identification and mitigation and (iv) proposal for additional tests predicting phenotypic behaviour and/or virulence.

Significance: Based on its new database and program, this phylogenetic affiliation tool facilitates accurate identification of isolates, such as emetic strains, anthracis-like strains or *B. thuringiensis* strains used as biocontrol agents. This tool further identicates additional tests to be performed if needed to identify hazard. This tool was developed thanks following the BtID national project, supported by the French ministry CASDAR program.

P1-162 Evaluation of BACARA® 2 Agar for the Detection and Enumeration of *B. cereus* Group

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Introduction: The *Bacillus cereus* group, including *B. cytotoxicus*, is a group of spore-forming strains causing diarrhea and emesis, which is associated with foodborne outbreaks and is a neglected human pathogen.

Purpose: We aimed to evaluate the performance of BACARA® 2 compared to BACARA®, MYP, and RAPID'*B. cereus*® to detect and enumerate *B. cereus* group in pure culture and foods.

Methods: We conducted inclusivity tests using *B. cereus* group strains (n=61) and exclusivity tests using non-*B. cereus* strains (n=31) on BAC2, BAC, RAPID, and MYP agars with TSA agar as reference medium. The plates were examined after 24 hours and 48 hours. Five foods matrices were selected, including liquid milk, whey powder, mashed potato, rice, and tea bag. There are five replicates per level per food matrix, including low, medium, high inoculation levels and negative control. Each replicate was diluted in a 1/10 BPB and 0.1 ml of the dilutions were inoculated on BAC2, RAPID, and MYP agars.

Results: In exclusivity tests, after 24 h incubation, 11 non-*B. cereus* strains grew on MPY but not on BAC2, BAC, or RAPID. Among these strains, three grew on BAC, three grew on BAC2, and two grew on RAPID after 48 hours. *Staphylococcus intermedius* and *Staphylococcus pseudintermedius* grew on all three media. In inclusivity tests, all six *B. cytotoxicus* tested positive in BAC2 and RAPID but only one grew on BAC after 24-hour incubation. Five grew on BAC after 48 h. BAC2 performed well for the detection of *B. cereus* strains compared to BAC and MYP.

Significance: We expect the use of BAC2 and/or RAPID agars improve the specificity and sensitivity of the BAM *B. cereus* method for both detection and enumeration, particularly *B. cytotoxicus*. In addition, the study will decrease the time to detect viable *B. cereus* strains without additional confirmation step compared to MYP.

P1-163 Development and Evaluation of Modified MPN Methodology for Enumerating Rifampicin-Resistant *E. coli* in Agricultural and Environmental Samples

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❖ ***Developing Scientist Entrant***

Introduction: Rifampicin-resistant *Escherichia coli* is commonly used in food safety research as a model microorganism. At low population levels, it is typically enumerated using the traditional Most Probable Number (MPN) method, which requires multiple days of incubation and suffers from a lack of specificity over rifampicin-resistant background flora. To enable detection using a simple color change and eliminate the need for subsequent agar plate confirmation, we developed and validated a modified MPN method based on lactose peptone broth (LPB) containing bromocresol purple and rifampicin that changes color based on pH shifts induced by lactose fermentation (a typical characteristic of *E. coli*).

Purpose: To compare the sensitivity, specificity and accuracy of our modified MPN methods with the traditional method in agricultural samples.

Methods: Rifampicin-resistant *E. coli* TVS353 was used as the target strain. The following bacterial suspensions (~1 log CFU/ml) were prepared in 1% peptone water: healthy and injured *E. coli* TVS353; *E. coli* TVS353 mixed with rifampicin-resistant *Salmonella* and non-rifampicin-resistant *E. coli*; *E. coli* TVS353 in soil homogenate with/without background flora; and *E. coli* TVS353 retrieved from previously inoculated spinach leaf surfaces. *E. coli* TVS353 was quantified by MPN in tryptic soy broth (TSB), TSB with rifampicin (TSBR), and LPB with rifampicin (LPBR), followed by plating on MacConkey agar with rifampicin for confirmation.

Results: At a predicted level of 1 log CFU/ml, there was no significant difference in the MPN index (9.3 to 46 cell/ml) of LPBR compared with TSB and TSBR in these suspensions (P>0.05).

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Significance: Lactose served as the only carbon source in LPBR, which selectively incubated *E. coli* over non-lactose fermenters. The color change enabled direct visualization of results instead of plating confirmation. The modified MPN method with LPBR is an effective tool to accurately enumerate rifampicin-resistant *E. coli* in multiple situations in agricultural and environmental studies.

P1-164 Integration of Swabbing Recovery and Optical Detection of Bacterial Cells on Food Contact Surface

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❖ ***Developing Scientist Entrant***

Introduction: Rapid and effective microbial detection on food contact surfaces is a critical step for ensuring food safety and public health. However, traditional plating practices are time-consuming and ATP bioluminescence methods are not specific to microbes.

Purpose: The objective of this study is to develop a rapid and effective method to recover and quantify bacterial cells on the food contact surface.

Methods: The method integrated the swab recovery and optical detection method previously developed. In brief, *Salmonella* as the model bacterial cells dried on a stainless-steel surface were recovered using 0.9 % NaCl pre-moistened foam swab and then released in 1/4 strength Ringer solution with tween 80 (1.5 %). The released cells were incubated with a pre-coated gold chip. The coating of the chip is 3-mercaptophenylboronic acids (3-MPBA) which have the selectivity to bind glycans on bacterial cell walls that enabled optical detection using a low magnification microscope (10× objective lens). Quantification of the cell is based on the number of pixels observed under the microscope.

Results: Compared to flocked nylon swabs, foam swabs showed an improvement in the percentage of bacterial release from 23.53±1.26 % up to 72.29±2.21 %. A further increase in the release rate with the help of ultrasound was achieved in nearly complete release (98.25±1.62 %). For the optical detection, the capture efficiency of 3-MPBA-coated gold chip for *Salmonella* was 61.25±1.04 % at 10^7 CFU/ml, showing largely higher than the bare one (5.95±0.23%). Moreover, the method achieved sensitive and statistically reliable detection of as low as 10^3 CFU/mL bacterial cells with great capability for quantification. The total analytical time for optical detection is approximately 2 h.

Significance: This study demonstrated a novel method for rapid and effective assessing of bacterial load on food contact surface in a low-resource setting for industrial applications.

P1-165 Effect of Buffer on Culture Bias in the Recovery of *Salmonella* Serovars from Mixed Cultures

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Introduction: *Salmonella* serovars are differentially recovered from enrichment broths, and illness-causing serovars such as Enteritidis and Typhimurium are masked by less severe serovars, such as Give and Kentucky. Improved media may aid the detection of important serovars from contaminated foods.

Purpose: Three different buffers were tested in peptone water for better recovery of disease-causing strains from mixed-serovar cultures.

Methods: Buffered Peptone Water was formulated with Phosphate, MOPS, and HEPES buffers. Four strains each of *Salmonella* serovars Give and Kentucky were co-cultured with four strains each of serovars Enteritidis and Typhimurium in the media. After overnight growth at 37°C, serial dilutions were plated and ≥30 colonies selected for typing. Colonies were differentiated by serogroup-specific antisera, and percent of each serovar recovered was calculated.

Results: Data was compiled from at least three independent replicates. Strains of serovars Kentucky and Give outgrew Enteritidis and Typhimurium in phosphate buffered medium in nearly all pairings. Kentucky colonies averaged approximately 80%, and serovar Give colonies 85% in pairings with Typhimurium and Enteritidis. Results were mixed in the MOPS and HEPES buffered media. In MOPS buffered medium, both Enteritidis and Typhimurium strains (12 to 35%) were outgrown by Give and Kentucky strains (65 to 83%). In most cases with HEPES buffered peptone water, Enteritidis and Typhimurium were detected less often. However, *S.* Typhimurium was recovered at statistically similar levels to *S.* Give strains when co-cultured in HEPES buffered medium (68% ± 16% Give vs 32% ± 16% Typhimurium). Additional pairings and buffers are being tested.

Significance: Accurate detection of disease-causing serovars such as Enteritidis and Typhimurium from foods and water naturally contaminated with non-disease causing serovars such as Give and Kentucky is essential for accurate *Salmonella* surveillance.

P1-166 Evaluation of Modern Outbreak Strains of *Salmonella* in an Immunodiffusion Assay: A Simple, Low-Cost, Effective Solution

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Introduction: This single-use test is an immunodiffusion assay for the detection of *Salmonella* in foods, food ingredients, and environmental samples. As *Salmonella* outbreak strains can differ based on food type, season, and global region, broad coverage in detecting *Salmonella* is crucial for public health and the prevention of foodborne illness. The ease-of-use, low cost, and global reach of the assay must be demonstrated with an ability to detect ever-changing outbreak strains.

Purpose: To demonstrate the most recent lot of 1-2 Test® Antibody Preparation will detect recent *Salmonella* outbreak strains.

Methods: The source of rabbits used to make the immunodiffusion assay antibody preparation is unchanged from previous manufacture lots. Sera was obtained from over 70 rabbits inoculated against various *Salmonella* species and screened to determine serogroup coverage. Using a proprietary mix of these sera, the antibody preparation was combined, purified, and titrated. This new lot of antibody preparation and a previous control lot were tested for specificity with an inclusivity and exclusivity panel of 106 strains (74 *Salmonella* and 32 non-target organisms) following the Directions for Use (DFU). Devices were examined after incubation for presence of an ImmunoBand™, indicating a positive result, and results were recorded as positive or negative.

Results: For both the new and control lots of antibody preparation, 72 of the 74 strains of *Salmonella* were detected. The two strains not detected were common between lots and not reported to be outbreak strains by the CDC between 2006-2022.

Significance: This study demonstrates the continued ability of the immunodiffusion antibody-based test to detect *Salmonella* strains from a large variety of foods, environments, regions, and recent outbreaks. Due to its ease-of-use and low cost per test, this assay remains an accessible and reliable option for testing products for *Salmonella* contamination.

P1-167 Evaluation of Modified Moore Swabs as a Concentrating Device for the Detection of *Salmonella* from Spent Sprout Irrigation Water

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Introduction: Routine testing of spent sprout irrigation water (SSIW) for foodborne pathogens ensures a safer supply of sprouts to United States' consumers. Sprout producers not within close proximity to testing laboratories must ship SSIW, incurring high shipping costs.

Purpose: To evaluate the use of modified Moore swabs (MMS) as a concentrating device for the detection of *Salmonella enterica* in SSIW.

Methods: Levels of detection (1, 2, and 5 CFU per 375 ml SSIW) were examined by comparing *Salmonella* detection from SSIW with or without filtration through MMS. A separate single-laboratory validation study, using two *Salmonella* serovars (*S*. Cubana and *S*. Harvana), was performed following FDA vali-

dation guidelines. Twenty SSIW test portions of unfiltered or MMS at low inoculation level (<1 CFU/375ml SSIW) were pre-enriched using universal pre-enrichment broth (UPB, 1:3 sample-to-broth ratio). FDA BAM culture methods were followed thereafter. Multiple rapid screening methods (VIDAS SLM, Roka Atlas and qPCR) were performed for a paired comparison with the culture method. Additionally, metagenomic samples were taken prior to enrichment, at 24 h (pre-enrichment) and 48 h (selective enrichments).

Results: The BAM method can detect *Salmonella* as low as 1 CFU/375 ml SSIW with or without MMS. Generally, incorporation of MMS showed no difference in the detection rate of *Salmonella*. In some cases, like inoculated with *S.* Cubana, incorporation of MMS even significantly improved *Salmonella* detection rate (0.55 with MMS filtration, 0.2 unfiltered). The *Salmonella* detection rate with selected rapid methods was comparable to culture method, regardless of filtration. A slight increase, though not significant, in the relative abundance of *Salmonella* in MMS quasi-metagenomic samples was observed. Additionally, culture independent MMS samples showed less low-abundant diversity than unfiltered samples.

Significance: MMS is a promising concentrating device for the detection of *Salmonella* from SSIW and may provide an easy, inexpensive, and reliable way to sample and ship SSIW for pathogen testing.

P1-168 Rapid Detection Method for *Salmonella* Infantis in Food Samples

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Introduction: The high prevalence of Salmonella in US poultry and recent requirements to develop a risk assessment approach and typing to reduce the serotypes of clinical relevance produces a need for rapid and reliable identification method for the Salmonella sub-species. Detection method for Salmonella Infantis in meats and poultry meats was developed to complement RapidFinder™ Salmonella Species, Typhimurium and Enteritidis Multiplex and SureCount™ Salmonella assays to track the three Salmonella serotypes commonly associated with human illness: Infantis, Enteritidis, and Typhimurium.

Purpose: The purpose of the study was to challenge the performance of the Salmonella Infantis assay with enriched food samples and determine the specificity of the assay by testing several Infantis strains along with other organisms to evaluate inclusivity and exclusivity.

Methods: Inclusivity analysis consisted of two parts, comparing the designed assay targets to NCBI GenBank Bacteria reference database and PCR analysis of S. Infantis isolates with QuantStudio™ 5 Real-Time PCR instrument. Panel of exclusion strains was constructed, and enriched food matrices were analysed to challenge the performance of the method as detailed in the manual.

Results: The inclusivity determination yielded in 98.8 % of inclusivity (in-silico sample size 17 660), confirmation with isolates (50 *S.* Infantis strains) indicated inclusivity of >95 %. Performance of the assay was confirmed with *S.* Infantis spiked raw chicken thighs, sausages and carcass rinses, POD results varied from 0.50-1.00 between samples and spiking levels demonstrating comparability between the test method and the reference method.

Significance: The study demonstrated that the Salmonella Infantis Kit offers reliable workflow for the rapid detection and specific identification of Salmonella Infantis from food samples.

P1-169 Comparison of the Modified Moore Swab (MMS) and Dead-End Ultrafiltration (DEUF) Methods for the Recovery of *Campylobacter* in Water

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Introduction: Several *Campylobacter* species cause human gastroenteritis worldwide, with infectious doses being as few as 500 bacteria. Campylobacteriosis is usually associated with consumption of raw milk, contaminated water, undercooked poultry, and seafood. Further, *Campylobacter* spp. have been isolated from soil, surface water, and groundwater, from which they may potentially be transferred to food crops. Most of the current methods for *Campylobacter* detection in water use small sample volumes, which may result in false negative results for water sources with lower contamination levels.

Purpose*:* To determine optimal concentration methods, namely Modified Moore Swab (MMS) and Dead-End Ultrafiltration (DEUF), for the recovery of *Campylobacter* from large volumes of water.

Methods: Ultrapure water (10L) was inoculated with various colony-forming units (CFU) of *Campylobacter jejuni* (*C. jejuni*) and processed using MMS or DEUF. For the recovery of *C. jejuni* from filters stored at 4°C, ultrafilters were pretreated with 5% fetal bovine serum (FBS) prior to water filtration to minimize bacterial adsorption by the filters. Each MMS was directly enriched in Bolton broth under microaerobic conditions at 37°C for 48 h and colonies were recovered on modified charcoal cefoperazone deoxycholate agar plates. Ultrafilters were backflushed with elution buffer and eluates were centrifuged at 8000 rpm. Recovered pellets were enriched as described above. In addition to PCR confirmation, colonies were screened for motility and *Campylobacter*-like morphology using a phase contrast microscope.

Results: Our results showed that the limit of detection was below 10 CFU/10 L water, for the MMS and DEUF methods. Further, we recovered *C. jejuni* from 5% FBS pretreated ultrafilters, that were stored at 4°C for 48 h.

Significance: The MMS and DEUF offer easy, rapid, and sensitive concentration methods for the recovery of low numbers of *Campylobacter* from large volumes of water. Further testing of these methods will be necessary for applicability to irrigation water sources.

P1-170 Evaluation of Growth in Four *Listeria* Enrichment Broths by Microbiome Profile Analysis Using 16S Metagenomics

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Introduction: Rapid detection of *Listeria monocytogenes* is important to prevent foodborne illness, including loss of life. Novel enrichment broths are developed for rapid detection of *Listeria* by single-step enrichment methods. 16S metagenomic profiling over different enrichment times can show changes in microbiome populations, illustrating growth promotion variations of enrichment media.

Purpose: Four proprietary *Listeria* enrichment broths were evaluated using 16S metagenomics profiles for its ability to grow *Listeria* monocytogenes in queso cheese.

Methods: Listeria monocytogenes was inoculated at log 1.08 CFU per 25-gram sample of queso cheese, in triplicate, for each of the *Listeria* enrichment broths, then incubated for 22 hours. Samples were analyzed at 0, 16, and 22 hours by NeoSeek™16S and cultural confirmations on Harlequin® *Listeria* Chromogenic Agar (LCA) at 16 and 22 hours. Samples were sub-cultured to Fraser broth and plated onto LCA for further verification.

Results: 16S metagenomic results revealed there were more than 22 different bacterial genera found in queso cheese. At time zero, *Bacillus* species levels were at around 32% and 62% for *Serratia* species; *Listeria* were undetectable before enrichment. After 16 hours of enrichment, only LESS Plus had any *Listeria* detected, at 0.13% relative abundance and continued enrichment to 22 hours increased *Listeria* levels to 1.2%. 24LEB was the only other broth at 22 hours with detectable levels of *Listeria* at 0.13%. LSB and LX broth each had undetectable levels even at 22 hours. Direct plating onto LCA at 16 and 22 hours supported the 16S relative abundance data by only recovering *Listeria* from LESS Plus enrichments and not the other three *Listeria* broths. Secondary enrichment in Fraser broth and plating to LCA confirmed that all enrichments broth samples indeed had inoculated *Listeria monocytogenes*.

Significance: Neogen's LESS Plus had the best performance out of four enrichment broths for detecting *Listeria* in queso cheese.

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P1-171 An Optimized *Listeria* Enrichment Media for 18-Hour Enrichment

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Introduction: *Listeria monocytogenes* is a foodborne pathogen that has been isolated from a wide variety of food matrices. Due to its slow-growing nature, laboratory testing involves long enrichment times and/or secondary enrichments, and the organism can also be outcompeted by high levels of background organisms.

Purpose: To develop and evaluate a primary enrichment medium capable of selectively enriching *Listeria* spp. in less than 20 hours for use with molecular detection platforms.

Methods: The proposed medium was tested for inclusivity by inoculation with *Listeria monocytogenes* (n=18) and other *Listeria* spp. (n=16) isolates and incubating at 37°C. To test performance with contaminated foods, eight different food matrices (raw milk, smoked salmon, bleu cheese, frozen spinach, salami, powdered milk, frozen mixed berries, and raw milk cheese) were artificially contaminated with *L. monocytogenes* or *L. seeligeri*. Organisms were heat stressed at 56°C prior to spiking into the food matrices. Spiked matrices were diluted 1:10 with prewarmed (37°C) media and incubated at 37°C. Samples were pulled at 18 and 24 hours and tested using BAC*Gene Listeria* spp. and *Listeria monocytogenes* PCR test kits. Results were culturally confirmed following ISO 11290-1:2017.

Results: All strains of *L. monocytogenes* and other *Listeria* spp. grew in the new medium, while common competing Gram positive organisms, such as *Enterococcus* and *Bacillus* spp. were inhibited. For all food matrices tested, *L. monocytogenes* and *L. seeligeri* were detected by PCR at both 18 and 24 hours and confirmed by culture methods.

Significance: These results demonstrate that BAC*Gro* ULTRA *Listeria* Broth (BULB) is a selective enrichment medium capable of producing shorter enrichment times than any commercially available method, allowing food processors and testing laboratories to achieve faster turnaround times.

P1-172 Inactivation of Shiga Toxin-Producing *Escherichia coli* O157:H7 (STEC), *Salmonella* and *Listeria monocytogenes* during Home Canning with Dishwasher Cycles

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Introduction: Home canning represents an economical method to preserve food with kitchen appliance at home when science-based recommendations are followed. However, unsafe canning methodologies are still preferred in practice.

Purpose: The purpose of this study was to evaluate the inactivation of shiga toxin-producing *Escherichia coli* O157:H7 (STEC), *Salmonella* and *Listeria monocytogenes* by dishwasher cycles used during home canning.

Methods: The 450 ml of peptone water, blended tomato (acidic non-viscous food) and potato puree (non-acidic viscous food) were prepared with 1.5% salt and 25 ml vinegar as model foods in glass jars. After sterilization, jars were inoculated with *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* at the levels of 10^6-10^7. The jars were treated with dishwasher cycles of Economic (50°C-122 min), Express (60°C-54 min) and Intensive (70°C-96 min). Population of surviving cells were enumerated by plate spreading on tryptic soy agar. Temperature changes in jars placed at top and bottom racks in dishwasher were monitored by using thermocouples.

Results: Measured temperature differences in jars were <1°C in both racks and tested locations in dishwasher during all cycle treatments (P>0.05). Dishwasher cycles reduced the pathogen populations the most in peptone water (>6 log) followed by blended tomato (2-6 log) and potato puree (<3 log) (P<0.05). *E. coli* O157:H7 was the most resistant in Economic cycle in tomato and potato (P<0.05). All pathogens showed similar resistance after Express cycle with a log reduction ranging from 4.2 to 5.0 log in tomato and 0.6 to 0.7 log in potato. In blended tomato, population reductions were between 4.6 and 5.7 log after Intensive cycle. Reduction in *L. monocytogenes* population was limited (0.6 log) compared to *E. coli* O157:H7 (2.0 log) and *Salmonella* (2.7 log) in tomato after Intensive cycle.

Significance: Insufficient dishwasher cycles to inactivate common foodborne pathogens during home canning shows unlikely inactivation of *Clostridium botulinum* as target organisms.

P1-173 Development of Spectrophotometric Method for Rapid Determination of Generic *Escherichia coli* Population in Agricultural Waters

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Introduction: Generic *Escherichia coli* is used as a reference indicator microorganism to monitor the microbiological quality of agricultural waters. Several methodologies are present to determine the population of generic *E. coli* in agricultural waters with various performances.

Purpose: The objective of this study was to develop an alternative method based on spectrophotometric measurement for the rapid determination of generic *E. coli* population in agricultural waters.

Methods: Chromogenic media (9 ml) was inoculated with two individual generic *E. coli* strains (ATCC 25922 and ATCC 35218) (1 ml) at concentrations of 10^0, 10^1, 10^2, 10^3, and 10^4. Tubes were incubated at 36 ± 1°C for 10, 12, 14, 16, 18, and 20 h (n=6). At each time interval, 1 ml of chromogenic media was transferred to microcentrifuge tubes for extraction of produced dye in chromogenic media. Absorbances of extracts (300 µl) were measured at 632 nm with a microplate reader. Correlation between absorbance and generic *E. coli* population was used to have prediction models after 10 and 12 hours of incubation. Predicted generic *E. coli* populations determined through the developed spectrophotometric model and traditional culture-based membrane filtration method (log CFU/100 ml) were compared for agricultural surface water samples from different sources.

Results: Absorbances were measured at similar ranges for both strains and inoculated concentrations at all tested time intervals. After extraction, high linear correlations were determined between actual *E. coli* populations and those calculated by models based on spectrophotometric reads at 12 hours (R^2=0.9195) and at 10 hours (R^2=0.8647). The correlation between the membrane filtration method and the developed spectrophotometric method used in the determination of the generic *E. coli* population was found to be moderately high (R^2=0.684) for agricultural water sources.

Significance: Performance of the developed spectrophotometric methodology seems promising to save time for the determination of generic *E. coli* population in agricultural water samples.

P1-174 Development of Method for Strengthening Hydrogen Bond between *Staphylococcus aureus* and Teicoplanin-Magnetic Beads

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Introduction: Use of magnetic separation improves *Staphylococcus aureus* detection, but the detection limit of the method still needs to be improved because of food matrix and low concentration of *S. aureus* in food.

Purpose: The purpose of this study was to develop a method to improve the capture efficiency of magnetic beads for *S. aureus* by strengthening hydrogen bonds between teicoplanin-magnetic beads and *S. aureus*.

Methods: A mixture of *S. aureus* strains ATCC14458, ATCC27664, ATCC13565, and ATCC23235 was prepared at 5.0 Log CFU/mL. Teicoplanin was attached to magnetic beads by an activation procedure, and concentrations of the teicoplanin-magnetic beads, incubation time, and separation time was examined to optimize the condition to detect *S. aureus*. In addition, sucrose (10%, 15%, and 20%; w/v), glycerol (20%, 25%, and 30%; w/v), or ethanol (3% and

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5%; w/v) were added to strengthen the hydrogen bond between teicoplanin-magnetic beads and *S. aureus*. *S. aureus* on the magnetic beads was detected by PCR and the concentrations of 1.0-5.0 Log CFU/mL were examined.

Results: Teicoplanin was successfully attached to magnetic beads. For the optimized magnetic separation condition for *S. aureus*, 40 µL of teicoplanin-magnetic beads, 45 min of incubation time at 180 rpm and 37°C, and 5 min of separation time were selected. Sucrose at 10% or ethanol at 3% were 87.3±4.6% or 84.5±5.1% of the capture efficiency, respectively. The detection limit was 3.0 Log CFU/mL when teicoplanin-magnetic beads were only used, while it decreased to 2.0 Log CFU/mL when 3% ethanol was mixed with the teicoplanin-magnetic beads.

Significance: These results show that the detection limit of teicoplanin-magnetic beads by adding 3% ethanol, and it might be caused by strengthening the hydrogen bond between *S. aureus* and teicoplanin-magnetic beads.

P1-175 Optimized Enrichment Protocols to Overcome *Salmonella* Growth Inhibition in Various Spices for Detection with Real-Time PCR

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Introduction: Spice matrices introduce a variety of difficulties for the detection of *Salmonella* contamination. One important consideration to research before testing these foods is whether enrichment protocols combined with the spice allow *Salmonella* to grow in order to avoid false negative results.

Purpose: In this study, we investigated various enrichment combinations of Buffered Peptone Water to overcome *Salmonella* growth inhibition in a variety of spices to allow detection using real-time PCR.

Methods: Spices including chili spice mix (garlic, high acidity, other spices), spicy and sweet seasoning mix (garlic, onion, other spices), seaweed spice mix (garlic, high acidity, other spices), vanilla spice mix (vanilla, salt compounds), ground ginger, and garlic powder were tested with a variety of enrichment protocols to determine the most sensitive and economical method.

Results: Successful growth of *Salmonella* was achieved in 24 hr with the chili spice mix (1x BPW, 1:300 enrichment ratio), spicy and sweet spice mix (1x BPW, 1:10 enrichment ratio, optional potassium sulfite additive), seaweed spice mix (2x BPW, 1:10 enrichment ratio, potassium sulfite additive), vanilla spice mix (1x BPW, 1:1000 enrichment ratio), ground ginger (1x BPW, 1:10 enrichment ratio, potassium sulfite additive), and garlic powder (1x BPW, 1:100 enrichment ratio, potassium sulfite additive).

Significance: These data show that *Salmonella* may not grow in standard enrichment conditions with some spices, but growth can be achieved with tailored enrichment protocols for detection with real-time PCR.

P1-176 Performance Comparison of 3M Petrifilm Rapid *E. coli*/Coliform Count Plate and ISO 16649-2:2001 Method for Enumeration of *Escherichia coli* in Processed Meat Matrices

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Introduction: Analyses of microbial contamination in production and finished products help demonstrate good processing practices. Coliform bacteria, specifically *Escherichia coli (E. coli)*, are useful as microbiological criteria to indicate contamination of processed foods. Thus, several agencies require analysis of *E. coli* in their guidelines and food regulations. Rapid methods to enumerate coliforms and *E. coli* for microbiological monitoring facilitate a quick response enabling efficient quality control which is beneficial to food industries.

Purpose: This study aimed to compare the performance of a rapid coliform and *E. coli* method and ISO 16649-2:2001 for quantification of *E. coli* in processed meat and poultry samples.

Methods: Commercial samples of beef hamburger, chicken nuggets, and turkey breast were used in the study. Ten samples of each matrix were artificially contaminated with *E. coli* ATCC 8539, *Enterococcus faecalis* WDCM 00087, and *Pseudomonas aeruginosa* ATCC 12924 at two levels (50 and 500 CFU/g). Samples were diluted ten-fold, followed by plating on sample-ready or traditional media. 3M Petrifilm Rapid *E. coli*/Coliform Plates were incubated at 37°C for 24 h while TBX plates were incubated at 44°C for 24 h. After incubation, the sample-ready medium was enumerated using an automated reader while TBX plates were enumerated manually. 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. P-values higher than 0.05 were obtained for enumeration of *Escherichia* coli in hamburger, chicken nuggets and turkey breast. 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 rapid *E. coli* and coliform method using a sample-ready medium enabled reliable enumeration of *E. coli* in processed food samples within 24 h, with results comparable to ISO 16649-2:2001.

P1-177 Evaluation of an Automated Reader for Improving Technician Time and Labor for Enumeration of Microbial Indicators in a Colombian Dairy Laboratory

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Introduction: Food safety and quality laboratories must generate reliable and rapid analytical results to meet increasing demand while working with limited resources such as time, space, and technicians. Tools that can help reduce turnaround times while providing accurate results may greatly improve laboratory efficiency.

Purpose: The time and method performance of an automated plate count reader for counting microbial indicator plates in dairy laboratory in Colombia was evaluated to demonstrate time savings while still maintaining comparable accuracy to manual enumeration.

Methods: Four 3M Petrifilm Plates (aerobic count, *Escherichia coli* (*E. coli*)/coliform count, *S. aureus* count, and *Enterobacteriaceae* count) were used in this study. Six dairy matrices were spiked at different levels, up to 550 colonies per plate, with of *E. coli* ATCC 25922*, Staphylococcus aureus* ATCC 25923, and *Klebsiella aerogenes* ATCC 13048. The time needed for plate labeling, data logging, and colony enumeration were measured for both manual and automated enumeration methods. Statistical differences were calculated using Student T Distribution ($\alpha=0.05$).

Results: The total time needed to complete reading, sample and data entry for the 120 plates was 83 minutes, 56 seconds and 38 minutes, 59 seconds for the manual and the automated enumerations methods, respectively. The automated reader provided a 54% reduction in time, with majority of time savings found within the colony enumeration step. There was no statistical difference between the colony count results for the two enumeration methods ($t_0=-0.1184$).

Significance: Automation can be a powerful tool to reduce time costs while providing accurate results for the enumeration of microbial indicators in dairy samples.

Poster

P1-178 NF Validation Study of a Chromogenic Agar Method for Enumeration of *E. coli* and Coliforms in Environmental Samples

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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 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 environmental samples category according to the ISO 16140-2:2016 validation protocol.

Methods: 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/ surface swabs, 2/ process water, 3/ dust and residue. 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 -0.03 log CFU/g (coliforms at 30°C) to 0.05 log CFU/g (*E. coli*). The standard deviation difference varied from 0.14 log CFU/g (coliforms at 30°C) to 0.16 log CFU/g (coliforms at 37°C). 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.

P1-179 Evaluation of Sample Rehydration Methods for the Enrichment and Recovery of *Cronobacter* in Powdered Infant Formula

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Introduction: Recent neonatal infections associated with *Cronobacter* in contaminated powered infant formula (PIF) calls for enhanced testing of *Cronobacter*. Previous research indicated that traditional 1:10 rehydration in enrichment broth may not be optimal for the recovery of *Cronobacter*, possibly due to the osmotic shock to desiccated *Cronobacter* cells. Thus, reduced rehydration could help improve the recovery of *Cronobacter*.

Purpose: This study compares several sample rehydration methods for the enrichment and recovery of desiccated *Cronobacter* cells in PIF.

Methods: Two types of PIF products were spiked with lyophilized *Cronobacter* at 0.8 CFU per 100 g test portion. Two categories of rehydration methods were compared to the rehydration method described in the FDA *Bacteriological Analytical Manual* (BAM), which is 1:10 rehydration in buffered peptone water (BPW) with mixing. One category of reduced rehydration methods maintained the final sample to BPW ratio at 1:10, 1) rehydration in 1:10 BPW with no mixing (i.e., soaking) and 2) rehydration in 1:3 BPW with mixing followed by addition of BPW to a 1:10 level 1 h later. The other category of reduced rehydration methods employed mixing at different sample to broth ratios, 1) 1:3 in BPW and 2) 1:5 in BPW. Samples were incubated and *Cronobacter* were subsequently isolated on chromogenic agars according to the BAM. Each method was performed with 20 replicates for each PIF product.

Results: The 1:3 rehydration for 1 h before addition of BPW that resulted in a final 1:10 rehydration produced higher recovery rate (Product-A 8/20, Product-B 14/20) than soaking (Product-A 3/20, Product-B 4/20) and BAM (Product-A 6/20, Product-B 11/20) methods. The 1:5 rehydration produced higher recovery rate (Product-A 8/20, Product-B 15/20) than 1:3 rehydration (Product-A 3/20, Product-B 13/20) and BAM (Product-A 3/20, Product-B 14/20) rehydration methods.

Significance: The data indicated that reduced rehydration could improve the recovery of desiccated *Cronobacter* cells in PIF.

P1-180 Sample Preparation Assessment and Validation of Microbial Contaminant Methods for a High-Load Yeast Matrix

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Introduction: Nestlé R&D utilized a proprietary, lyophilized strain of *Pichia kluyveri* (10^6 CFU/vial) to produce flavor compounds that will be applied to food products. However, no data is currently available on the use of established food methods that would be capable of detecting contaminants.

Purpose: The objectives of this study were two-fold: 1) conduct sample preparation assessments to determine the appropriate enrichment dilutions for microbiological testing, and 2) determine if methods for *Enterobacteriaceae* (ISO 21258-1:2017), *Salmonella* (ISO 6579-1:2017), and coagulase-positive Staphylococci (ISO 6888-3:2003) were fit-for-purpose for contaminant detection in the yeast matrix.

Methods: Yeast vials were aseptically opened, rehydrated with yeast-peptone-dextrose (YPD) broth, and allowed in incubate at 30°C for 30 minutes. Sample preparation assessments were conducted to determine the appropriate enrichment dilution in 6-fold strength buffered peptone water (6S BPW) for EB and *Salmonella*, and GC broth for coagulase-positive Staphylococci. The limit of detection at 50% (LOD_{50}, n=30) was determined for each contaminant organism in the presence of the yeast matrix, as specified in ISO 16140-2:2016.

Results: A 1:10 enrichment dilution in 6S BPW was sufficient for detection of EB and *Salmonella*. However, a 1:100 dilution in GC broth was needed for Staphylococci as interference from the yeast impeded detection at lower dilutions. The LOD_{50} values were 0.289, 0.187, and 0.489 CFU/vial for EB, *Salmonella*, and coagulase-positive Staphylococci, respectively.

Significance: The ISO methods were fit-for-purpose and sensitive for detection of microbial contaminants in the yeast matrix.

P1-181 Evaluation of Enrichment Broths Used for the Detection *of Escherichia coli* O157 in Dairy Products

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Introduction: The mTSB-n enrichment broth is required for the detection of *Escherichia coli* O157 in the cultural reference method of Health Canada's Compendium of Analytical Methods. Results obtained in a previous project and found in Scientific's literature has demonstrated that this broth might not be the most efficient for the recovery of *E. coli* O157:H7 in some foods.

Purpose: The purpose of this study was to evaluate the performance of various enrichment broths proposed in the literature or used in different methods of regularity bodies for the detection of *E. coli* O157:H7 in the dairy products.

Methods: Selectivity was performed over a 24 h period with 3 strains of *E. coli* O157 with or without freezing stress using mTSB-n, mEPTp, EPT, BRILA and SSSLE broths. Two broths were then removed: SSSLE (low growth) and EPT (non-selective). LOD (Limit of Detection) was performed on a cheese made from raw milk. POD (Probability of detection) was performed on 3 types of foods: raw (cheeses made from raw milk), heat-treated (milks) and fermented (kefirs).

Results: The LOD and POD of the mEPTp broth demonstrated significantly higher detection capability for cheeses made from raw milk compared to the mTSB-n reference broth. For heat-treated dairy products, the mTSB-n/mEPTp broths demonstrated the same performance. It is imperative not to substitute mTSB-n broth with mEPTp broth for the detection of *E. coli* O157 in fermented dairy products such as kefir, since the POD is significantly reduced upon enrichment in mEPTp broth.

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Significance: This project demonstrates the importance of the choice of the enrichment broth for the detection of a pathogen in food and supports CFIA's efforts to develop a new O157 cultural reference method.

P1-182 Atypical Hemolytic *Listeria* Isolates May be Misidentified with Rapid Detection and Identification Methods

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Introduction: Commercial methods for rapid detection and identification of *Listeria monocytogenes* often target the virulence traits associated with this species (e.g., b-hemolysis). Hemolytic *L. innocua* share several virulence traits with typical *L. monocytogenes,* while non-hemolytic *L. monocytogenes* often lack these characteristics.

Purpose: The purpose of this study was two-fold. First, evaluate the potential for *L. monocytogenes* rapid detection methods to yield (i) false positives with hemolytic *L. innocua,* and (ii) false negatives with non-hemolytic *L. monocytogenes*. Second, evaluate if rapid identification methods will correctly speciate atypical hemolytic *Listeria* isolates.

Methods: A strain set consisting of (n=3) non-hemolytic *L. monocytogenes* and (n=3) hemolytic *L. innocua* was utilized in this study. For the rapid method analyses, pure broth cultures were prepared and diluted to the method limit of detection plus 1 $\log_{10}$ and tested per the manufacturer's instructions. The rapid method evaluation included PCR (n=4) and antigen-antibody (n=1) methods. The rapid identification methods were tested from growth on nonselective agar per the manufacturer's instructions. The rapid identification methods included biochemical (n=3) and MALDI-TOF MS (n=2) methods.

Results: The hemolytic *L. innocua* isolates generated *L. monocytogenes* PCR-positive results with two of the four PCR-based methods and the antigen-antibody method; all assays detected the non-hemolytic *L. monocytogenes.* Both the MALDI-TOF identification methods correctly identified the *Listeria* species tested. The biochemical methods showed a high potential for misidentification, but this potential varied by isolate and method.

Significance: Atypical hemolytic *Listeria* isolates may yield an *L. monocytogenes* false positive or false negative depending on which detection and identification methods are employed. From this study we identified the importance of the D-arylamidase and Phosphoinositide phospholipase C activity tests in differentiating an atypical hemolytic *L. innocua* from *L. monocytogenes.*

P1-183 Beer Spoilage Microorganisms: Molecular-Based Assays for Discrete Contamination Detection

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Introduction: Breweries face challenges on detection and avoidance of beer spoilage because contamination, when present, shows a discrete pattern and may be caused by a number of spoilers such as wild yeasts, lactic acid bacteria and, more rarely, *Megasphaera* or *Pectinatus*.

Purpose: Evaluate the molecular based approach for detection of spoilers versus traditional techniques at different stages of production.

Methods: Fermentation vessels samples (n=10) were collected to assess contamination with viable bacterial cells (coupled detection of lactic acid bacteria, *Megasphera*, *Pectinatus* and hop resistance genes) in comparison with culturing on NBB-C. Semifinished product samples (n=10) were collected after filtration process to assess contamination with bacterial enrichment of membrane on MRS followed by coupled detection of lactic acid bacteria, *Megasphera*, *Pectinatus* and hop resistance genes versus NBB-A technique – and yeast cells – enrichment of membrane on brewSTAT followed coupled detection of *S. cerevisiae* diastaticus, *Dekkera* and *B. bruxellensis* versus YM tecnhnique).

Results: Results were gathered on three groups being (1) fermentation vessels samples, (2) bacterial detection on semifinished products and (3) yeast detection on semifinished products. Results for each group, each technique and each analyte (molecular or traditional) were contabilized and classified by being positive and negative, allowing a Chi-Square analysis for each criteria. For all scenario, no significant difference has been detected on the 95% interval of confidence, showing equivalence between traditional and molecular methods.

Significance: GENE-UP Brew Pro approach has shown to be a reliable method for spoilage assessment at the brewery, with the advantage of being faster than traditional methods. Early warnings related to the hop resistance gene detection were also observed, as one cell may show more than one copy of the gene, but they stood as statistically not significant, probably due to the number of samples.

P1-184 Identification of Signature Near Infra-Red Wavelengths to Predict Level of Food Spoilage Using Big Data Analytics Methodology

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❖ ***Developing Scientist Entrant***

Introduction: Rapid food spoilage detection technologies can reduce food waste and food loss but need to be optimized.

Purpose: The objective was to identify near infrared (NIR) signature wavelengths to predict spoilage of raw chicken breast fillets.

Methods: Commercial tray-packs of boneless, skinless chicken breast fillets placed in walk-in cooler (4˚C) were sampled on alternate days until deemed spoiled (>7 log CFU/ml). Fillets were analyzed using a portable near infrared spectroscopy device (350-2500 nm) to collect reflectance at each wavelength at 6 different positions on each fillet (n = 210 fillets, 2151 data points x 6 positions) before and after tray packs were opened. Collected NIR wavelength data was subjected to Andrew Curve Analysis before and after data preprocessing for visualization of multidimensional data for data complexity and overlapping before feature extraction of specific signature NIR wavelength associated with each stage of spoilage curve. Feature extraction of pre-processed data was performed using cluster variable and predictor analysis. Fillets were also analyzed for aerobic plate counts and used to train the Back propagation Neural Network (BPNN) (250000 steps, learning rate 0.02 and 5 hidden layers) for spoilage classification into baseline microbial count (up to 3 log CFU), propagation (3-6.9 log CFU) and spoiled (> 7 log CFU).

Results: Feature extraction techniques extracted signature wavelengths of 385 nm, 400 nm, 432 nm, 1141 nm, 1321 nm, 1374 nm, 2241 nm, 2292 nm, 2311 nm, 2412 nm for the separation of spoilage phases. The BPNN classification model were able to classify the microbial growth at different stages of spoilage in fillets with classification accuracy of 93.7 % for baseline counts, 90.0 % for propagation, and 88.9 % for spoiled.

Significance: Signature NIR wavelengths can be used to develop affordable rapid spoilage of perishable food products.

P1-185 Engineered Yeast Displaying Specific Norovirus-Binding Nanobodies for the Concentration and Detection of Human Norovirus in Food Matrix

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Introduction: As the leading cause of foodborne illness worldwide, human noroviruses pose grave threats to public health and the global economy. The major drawback of existing detection methods is that they require human noroviruses to be concentrated and purified from complex samples for reliable and sensitive detection.

Purpose: Herein, we aimed to genetically engineer yeast (*Saccharomyces cerevisiae* EBY 100) to display specific norovirus-binding single-domain antibodies (nanobodies) Nano-26 and Nano-85 on cell surface to facilitate concentration and purification of noroviruses for improved detection.

Methods: The Aga2p-HA-nanobody fusions were integrated into *S. cerevisiae* genome by homologous recombination where Aga2p serving as anchors to link Aga1 proteins on the whole-cell surface through two disulfide bridges. The engineered yeasts displaying Nano-26 and Nano-85 on cell surface were named as EY-NB-26 and EY-NB-85, respectively. Wild-type yeast and yeast displaying peptide CD20 from pCTcon2 instead of nanobody on the surface was

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used as negative and positive controls, respectively. Binding of norovirus virus-like particles (VLPs) to these nanobody-displaying yeasts was confirmed and characterized using confocal microscopy and flow cytometry.

Results: The ability of our engineered yeasts to capture norovirus VLPs was assessed using a bicinchoninic acid assay and the maximum capture efficiency using Nano-26 and Nano-85 can reach up to 91.3% and 74.3%, respectively. Furthermore, we have used this approach to concentrate and detect norovirus VLPs in a real food matrix using a sandwich assay. A wide linear detection range (10^1 to10^5 pg/mL; R^2 = 0.9634) was observed and the detection limit of norovirus VLPs on spiked spinach leaves was calculated as low as 0.86 pg/ml using EY-NB-26.

Significance: Overall, our engineered yeasts could be a promising approach to concentrate and purify human noroviruses in food samples for easy detection, which allows to prevent the spread of foodborne virus in the food supply chain and reduce the risk of foodborne outbreaks.

P1-186 Evaluating the Ability of Magnetic Ionic Liquids to Concentrate Human Norovirus Surrogate from Matrices Containing Potentially Interfering Charged Species

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Introduction: Magnetic ionic liquids (MILs) are a class of hydrophobic solvents that have previously shown promise as capture reagents for non-enveloped viruses in aqueous suspension. However, their binding is charge-based and may be susceptible to interference from food matrix components. Therefore, it is important to evaluate their binding affinity in the presence of interfering charged species.

Purpose: The purpose of this study was to evaluate the ability of MILs to concentrate bacteriophage MS2, an accepted human norovirus surrogate, from suspensions containing potentially interfering charged species at different concentrations.

Methods: MS2 was diluted to 10^5 PFU/mL in 1X PBS with varying concentrations of charged species and concentrated using a dysprosium (III)-based MIL. Briefly, MIL was added to the suspension, vortexed to disperse the MIL droplets and separated using a magnet. Supernatant was removed and samples were washed to remove unbound MS2. Captured MS2 was eluted by vortexing with modified LB broth, then viral ssRNA was purified and quantified by RT-qPCR.

Results: A charged species mixture consisting of 0.025% bovine serum albumin (BSA), 0.035% yeast extract, and 0.008% porcine gastric mucin led to a <0.3 log reduction in recovered MS2 compared to buffer-only suspension (change from 10.6±2.41% to 5.6±61.14% recovery, n=3). Next, BSA was added at higher concentrations to the MS2 suspension. About 1.4 log MS2 was recovered in the presence of 0.2% BSA, compared to 2.2 log without BSA (from 9.55±3.72% to 1.45±0.34% recovery, n=3). An increase in MIL dispersion was observed in the presence of charged species, which may have helped counteract some of the negative effects.

Significance: These results indicate that MILs can still recover significant amounts of non-enveloped virus target in the presence of interfering charged species, suggesting potential for their use to concentrate viruses from food and environmental samples.

P1-187 Method Comparison for Human Norovirus Concentration and Molecular Detection in Wastewater

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❖ ***Developing Scientist Entrant***

Introduction: Wastewater-based epidemiology has emerged as an important tool for assessment of viral load and diversity at the community level; however, this complex matrix poses challenges for molecular detection due to often low levels of target and high abundance of non-target genetic material, necessitating some form of pre-analytical sample preparation.

Purpose: The purpose was to compare the recovery efficiency of three different wastewater concentration and extraction methods for downstream molecular detection of spiked or naturally contaminated samples with a norovirus (NoV) surrogate and NoV, respectively.

Methods: Wastewater samples were pasteurized at 60ºC for 60 minutes and spiked to a concentration of 2 x 104 pfu/mL of MS2 bacteriophage. Spiked samples were incubated at 4ºC for 90 minutes for equilibration of virus into the solid and liquid fractions of the sample. Three different nucleic acid extraction/concentration protocols were employed, including PEG precipitation coupled with the Qiagen Viral RNA mini kit, Promega Wizard Enviro Total Nucleic Acid Kit, and Ceres Nano Nanotrap Microbiome A particles with the Promega Maxwell HT Environmental TNA kit. Samples collected over a 3-month time span (n = 40) were analyzed by digital PCR for spiked MS2, naturally present NoV (GI + GII), and Pepper Mild Mottle Virus (PMMoV).

Results: Capture efficiencies ranged from 0.6% (SD ± 0.62) to 19.57% (SD ± 17). The highest recovery efficiency of MS2 was observed for the nanoparticle method, with significantly higher recovery than the PEG and Wizard protocols (p = 0.0009 and 0.0005). PMMoV was quantified at a significantly higher level for the nanoparticle method compared to the other two methods (p = 0.008 and 0.002), and NoV was not significantly different among the methods (p = 0.1 and 0.09).

Significance: The choice of method for viral recovery in wastewater can significantly impact detection, ultimately impacting the effectiveness of wastewater epidemiology to inform public health measures.

P1-188 Field Validation of Microsnap™ Surface Express Total (MSX-Total) Using 6-Hour Rapid Surface Microbiology Detection Swab

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Introduction: This study demonstrates the use of MicroSnap™ Surface Express Total (MSX-Total) in the field and detection levels and time to result.

Purpose: Improve detection of surface total viable count (TVC)through a simple swab system that allows same shift detection in as short as 4 hours.

Methods: A total of 300 surface swabs were collected from local kitchens and lunchrooms. 100 sample swabs were incubated for 4 hours, 5 hours and 6 hours, respectively, at 32°C. Prior to activation of each swab at 4, 5 and 6 hours, an aliquot was removed from liquid media in each swab and plated onto TSA to assess the relationship between media growth as measured by bioluminescence and plating confirmation. Once activated, each swab was then read in a luminometer and the RLUs recorded, these RLUs were proportional to the starting inoculum swabbed at time 0.

Results: After 4 hours incubation, 16% of the swabs were positive for growth with RLUs ranging from 0 to 20,000 RLUs. The threshold RLU between no growth and growth was 30 RLUs; this RLU equated to a mean CFU of 250 and a PoD% of 100%. After 5 hours incubation, 27% of the swabs were positive for growth. The threshold RLU between negative growth and growth was 30 RLUs; this RLU equated to a mean CFU of 50 and a PoD% of 98%. After 6 hours incubation, 27% of the swabs were positive for growth. The threshold RLU between negative growth and growth was 30 RLUs; this RLU equated to a mean CFU of 10 and a PoD% of 70%. This was lower due to fractionality.

Significance: Surface TVC can be performed rapidly and with a high degree of confidence using the MSX Total swab.

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P1-189 Performance Evaluation of Three Rapid Microbial Indicator Enumeration Methods a Food Laboratory in Bogota, Colombia

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Introduction: Microbial enumeration methods must be demonstrated as fit for purpose before routine testing implementation in food safety and quality laboratories. The recent ISO 16140-3:2021 standard for method verification enable the generation of data for fully validated methods to be implemented within the scope of a laboratory, providing information related to the proficiency of the laboratory to perform the method and whether the method is fit for purpose for a specific application.

Purpose: In this study, the performance of three different rapid microbial enumeration methods was verified for analyses of four food categories in an analytical laboratory in Bogota, Colombia following the ISO 16140-3:2021 standard.

Methods: The performance of three 3MPetrifilm Plate methods (rapid *E. coli*/coliform, *Staphylococcus aureus* and *Enterobacteriaceae*) were evaluated with four different food categories. The following matrices were used in the study: two food items under raw milk and dairy products; one item under eggs and egg products; three items under dried cereals, fruits, nuts, seeds, and vegetables; and one food item under chocolate, bakery products and confectionary categories. Three test strains *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Enterobacter cloacae* subsp. *cloacae* ATCC 35030 were used to spike the different samples at three inoculum levels: 3-5, 140-180, and 800-900 CFU/g. Interlaboratory reproducibility (S_{IR}) and eBias were determined following ISO 16140-3:2021 for method verification.

Results: For method implementation verification, using chia negra as the test, the S_{IR} values obtained for *E. coli*, *S. aureus,* and *E. cloacae* were 0.195, 0.157, 0.179, respectively. For all the food samples, the methods included in the verification study (rapid *E. coli*/ coliform, *Staphylococcus aureus,* and *Enterobacteriaceae*) showed an eBias lower than 0.5 $\log_{10}$ CFU/g for all inoculation levels.

Significance: All three test methods evaluated for four different food categories met acceptance criteria and were considered verified for implementation according to ISO 16140-3:2021.

P1-190 The Combination of Filtration and ATP+ADP+AMP Assay for the Assessment of Microbial Quality of Water

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Introduction: Water microorganisms are a useful indicator for the quality of drinking water, and for the maintenance of water treatment facilities and circulating cooling water systems. Since culturing methods take several days, a rapid assay is required for quick response measures for incidents.

Purpose: A rapid test for microorganism-derived total adenylates (ATP+ADP+AMP, A3) was developed using a combination of the A3 test and filtration of water sample and eliminating the need for culture. The correlation between microorganism-derived A3 and viable bacterial counts was evaluated in this project.

Methods: Eight kinds of samples were collected from tap water, well-water, utility waste water, and cooling tower water. These samples were stored at 25 °C for 0-18 days to assess the change of microbial quality. Each sample (10 mL, total 29 samples) was filtered through a syringe filter to eliminate extracellular A3 and trap microorganisms. Successively, microorganism-derived A3 was extracted by the surfactant. The extract was measured by the A3 test (LuciPac™ A3 Surface/Lumitester™ Smart, Kikkoman Biochemifa Company), and the measurement output was relative light units (RLU). Colony counts were measured as colony forming units (CFU) and determined using R2A agar medium after 48 hours of incubation at 30 °C.

Results: During the sample storages, RLU values and log CFU increased as time passed. In 4 samples, bacteria and RLU values decreased at the end. The measurement values for all samples were 1.2-4.1 log RLU and 2.2-6.3 log CFU/mL. A positive correlation was found between RLU and CFU/mL in logarithmic scale ($y = 0.622x - 0.147$, $r^2 = 0.831$).

Significance: It was demonstrated that the microbial quality of water can be assessed using a quick (ca. 3 min) test and simple procedures - the combination of filtration treatment and A3 test. These results indicated that this assay is useful for rapid hygiene control of water and water-related facilities.

P1-191 Review of Good Laboratory Practices (GLPs) Associated with Microbiology Methods to Ensure Reliability of Pathogen Testing

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Introduction: GFSI, customer, and regulatory oversight on the robustness of third-party laboratory testing for pathogens has progressively increased over the years. A subsection of the IAFP Applied Laboratory Methods Professional Development Group collected data on best practices in microbiological testing.

Purpose: Pathogen testing has been the fastest growing category in the food microbiology contract labs since the past decade. With the high cost and impact to the food industry, it is imminent that the laboratories adhere to best practices, as stipulated by third-party certifications and FDA recommendations.

Methods: Two rounds of surveys were sent to third-party laboratory and industry personnel to obtain information on general awareness of GLPs. The IAFP Program Coordinator assisted with the data collection process. The results were compiled as bar graphs with a section provided for comments to obtain additional data on best practices for pathogen testing in the food microbiology labs.

Results: The case study was reviewed for a participant sample size of 18, over 9 questions. Labs' protocol for receiving samples & sponges, indicate temperature, condition, and transit time, as combined significant parameters (60% for respondents) for samples' integrity. Labs use productivity, sterility & specificity of the media, as the gold standard for performance. However, shelf life (76% of respondents) and storage temperature (53% of respondents) were considered as the top 2 parameters in ensuring optimal media performance. Despite some shortcomings (0% of respondents considered water-bath sanitation critical for melted media and only 13% expressed familiarity with FDA guidelines on enrichment media incubations), overall, participants submitted comments regarding following laboratory best practices by adhering to established SOPs.

Significance: The goal of this IAFP working group is to develop a review paper, using the knowledge obtained from the surveys. Recommendations, from resources such as ISO, AFNOR, and FDA's best practices manual will be used to highlight the gaps that should be addressed to ensure the robustness of pathogen testing in foods and environmental samples.

P1-192 Considerations When Implementing Food Microbiology Methods for Routine Testing: Thoughts from a Canadian Regulator's Perspective

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Introduction: As a regulator, choosing a method to implement in food microbiology testing laboratories is a complex decision that involves many factors.

Purpose: Present the Canadian Food Inspection Agency's (CFIA) approach when implementing test methods for routine microbiological analysis of foods to support regulatory decision making.

Methods: With more than 6,000 employees and 13 laboratories across Canada, the CFIA is Canada's largest science-based regulatory agency and is dedicated to safeguarding food, animals and plants, which enhances the health and well-being of Canada's people, environment and economy. Surveillance of the food supply is one of many CFIA controls that supports the above mission. Approximately 25,000 samples are tested per year to support the CFIA's microbiology surveillance programs and food safety investigations. Methods used to test these samples must have demonstrated equivalence to Canadian Reference methods and should be cost effective, scalable, reliable and ideally offer fast turn-around-times to final results.

Results: Prior to implementing new methods or platforms in its laboratories, the CFIA performs additional validation studies. This allows the CFIA to have additional confidence that the method will perform as expected with a broad range of commodities. This approach also leads to the optimization of published protocols (addition of matrices, consideration for various ingredients and addition of optional procedures to be used in certain scenarios, for example).

Significance: The CFIA considers method performance, applicability, cost and ease of use before implementing a new method. The approach taken by the CFIA ensures confidence in the results generated to support regulatory decision making. Depending on the testing scenario, there may not be a single method that can meet the requirements of all samples under all circumstances.

P1-193 Determination and Consumption Risk Assessment of U.S. EPA and EU Priority Polycyclic Aromatic Hydrocarbons (PAHs) in Coffee Samples Prepared Under Different Conditions

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Introduction: Polycyclic aromatic hydrocarbons (PAHs) are genotoxic and carcinogenic. PAHs in food may be transferred from PAHs-contaminated air, soil and water. Food processing also contributes to the formation of PAHs in food. The U.S. Environmental Protection Agency has recommended monitoring 16 PAHs (US EPA PAHs) commonly found in environmental samples. The European Food Safety Authority has defined 16 priority PAHs (EU priority PAHs) for food surveillance.

Purpose: Development of appropriate methods for simultaneous determination of US EPA and EU priority PAHs in coffee samples to assess the effect of roast degree on PAHs in coffee beans, the effect of brewing method on the transfer of PAHs from coffee beans to their brews, and drinking risk of PAHs in the coffee brews.

Methods: The coffee beans of different roast levels were prepared, and then brewed separately with the drip bag and the coffee machine. The optimized QuEChERS conditions were used to extract the PAHs from the samples. The developed conditions of high-performance liquid chromatography coupled with temperature controllable fluorescence detector and gas chromatography coupled with tandem mass spectrometer were used to analyze the PAHs. The coffee consumption recorded in the Taiwan Food Consumption Database was used to assess the PAHs dietary risk.

Results: PAHs could be measured in commercially available green coffee beans (28.85 ng/g), and their PAHs content increased with the degree of roasting (127.40-822.26 ng/g). Coffee beans brewed with the coffee machine released more PAHs into their brews (1.20-2.91 ng/g) than those brewed with the drip bag (0.72-1.96 ng/g). Because of the low PAHs content in the coffee brews, their PAHs dietary risk was not high.

Significance: The established methods allow for a more accurate assessment of PAHs in coffee samples and their consumption risk. The results could provide a reference for reducing PAHs intake in coffee preparation.

P1-194 Development of Multi-Residue Analytical Method for 189 Pesticides in Livestock Products Using LC-MS/MS and GC-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. 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 189 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) and gas chromatography-tandem mass spectrometry (GC-MS/MS)

Methods: Residual pesticides were extracted with acetonitrile followed by addition of $MgSO_4$ and sodium chloride. Then, the extracts were cleaned up using $MgSO_4$, C_{18} and primary secondary amine (PSA). To verify the applicability of the developed method, selectivity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) were evaluated.

Results: The coefficient of determination (R^2) was above 0.98 within the concentration range from 2.5 to 100 μg/L. The average recoveries were most in range of 70~120% and standard deviation values were less than 32% at spiked levels of 0.01, 0.02 and 0.1 mg/kg which is satisfied the Codex 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.

P1-195 Insects in My Food? Can Target Sequencing be Used to Detect and Identify Insects in Food Samples?

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Introduction: Identification of insect fragments provides valuable information for differentiating avoidable vs. unavoidable filth in food samples. However, insect fragment identification is not always possible using current microscopic morphological approaches. Target enrichment via hybridization-based capture is becoming a powerful next-generation sequencing tool to increase sensitivity of detection of low amounts of target DNA from complex food backgrounds. Also, when used alongside reliable taxonomy databases it provides accurate level of identification of target organisms.

Purpose: Evaluate target enrichment using hybridization-based capture with insect-specific baits to increase sensitivity of insect detection and identification in food samples.

Methods: A target capture kit containing insect-specific hybridization baits with mitochondrial probes from about 2200 insect species was custom designed. Insect fragments from a storage insect pest were used to spike wheat flour at 8 levels (0, 1, 2.5, 5, 7.5, 10, 100, and 1000 ppm), five replicates per spiking level (n=40). Genomic DNA was extracted from 10 g of spiked samples and then fragmented using the KAPA HyperPlus Kit. Pre- and post-target capture libraries were amplified and sequenced on the Illumina Miseq system. Sequencing data were analyzed with the MitochonTrakr pipeline to estimate the relative abundance of sequence reads and the four-parameter logistic analysis was further used to calculate limit of detection (LOD) in pre- and post-target capture data.

Results: Relative abundance of spiked insect fragments ranged 0.1%±0.07 to 62%±2.5 and 16%±1.4 to 99%±0.05 in pre- and post-target capture samples, respectively. While target capture decreased non-specific reads it increased insect reads in non-spiked samples (0 ppm) by 8.4%±1.17. Target capture increased LOD by 3.2-fold, from 30.7 to 9.7 ppm.

Poster

Significance: Target capture using insect-specific baits and the MitochonTrakr pipeline, successfully increases sensitivity of detection of low levels of insect fragments in food samples and provides accurate level of insect identification.

P1-196 Deveolpment of Analytical Method and Measurement of Mycotoxins from Retail Food

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Introduction: Mycotoxins are the second metabolites produced in fungi growing on agricultural products. Trichothecene (TCT) is a mycotoxin produced mainly by fungi of the genus *Fusarium*. TCT is categorized into 4 types: A, B, C, and D.

Purpose: We investigated the concentration of type A toxins including T-2 toxin, and HT-2 toxin, and type B toxins including deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), and 15-acetyldeoxynivalenol (15-ADON) in retail food.

Methods: We developed an analytical method using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for determination of five TCT from food matrices. Uniformly labeled [13C] analogues of the target analytes were used as internal standards and the samples were extracted with 84% acetonitrile followed by purification with a solid-phase extraction cartridge.

Results: The method validation was performed on four representative food matrices: coffee, chestnut, apple, and rice. Accuracy, precision, and specificity of this method were satisfied with the criteria of AOAC validation guidance. With the developed method, 5 TCTs were measured from 1,130 food samples out of 112 food categories.

Significance: This developed method and data will be used for evaluation of risk by diet of food and for the safety of food.

P1-197 An Automated Next-Generation Sequencing Method for Simultaneous Detection and Serotyping of *Salmonella* Directly from Enrichments

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Introduction: The proposed USDA regulatory framework to reduce *Salmonella* illness recognizes the importance of identifying the *Salmonella* serotypes more commonly associated with poultry and human illness, but also recognizes the difficulties presented by the lack of available, rapid, affordable serotyping methods.

Purpose: The purpose of this study is to evaluate an automated, high throughput NGS assay that can simultaneously detect and serotype *Salmonella* from sample enrichments.

Methods: In this study, 103 reference strains representing 78 *Salmonella* serovars were grown on tryptic soy agar incubated at 35°C overnight. Isolated colonies as well as mixed cultures were tested with the Clear Safety™ *Salmonella* method. In addition, 30 mL of chicken carcass rinsate were inoculated separately with 4 CFU of the most common serovars Enteritidis, Kentucky, Newport, Typhimurium, Javiana, I 4,[5],12:i:-, Infantis, Muenchen, Montevideo, Braenderup, Thompson, Saintpaul, Oranienburg, and Mississippi to simulate real-world food samples. They were enriched with 30mL *Salmonella* enrichment media with 20 mg/L novobiocin, incubated for 24 h at 35°C, and tested on the automated NGS platform.

Results: All 103 pure reference cultures of *Salmonella enterica* subsp. *enterica* were correctly identified and serotyped on the automated NGS platform. Top-14 *Salmonella* were detected and serotyped correctly in 14/14 carcass rinse sample enrichments. Samples co-infected with any two combinations of serovar Enteritidis, Kentucky, Newport, Typhimurium, I 4,[5],12:i:-, and Infantis were correctly identified except for the combination of Typhimurium and I 4,[5],12:i:- serovars. This NGS method identified serovars accurately with 100% sensitivity and 100% specificity as a pure culture and in the presence of carcass rinse background.

Significance: This NGS-based automated platform is suitable for simultaneous detection and serotyping of *Salmonella* in food products with high specificity and sensitivity directly from enrichment, reducing the time and labor to isolate colonies, and, at the same time, capable of meeting the proposed mandatory serotyping requirement from USDA-FSIS.

P1-198 Tahini and *Salmonella* – A Perfect Pairing!

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Introduction: Tahini is a popular, low-moisture, RTE food product; it is presumed to be free from pathogens. However, in the recent years (2018 and 2022), Ontario has had three reported *Salmonella* outbreaks associated with exposure to tahini.

Purpose: The purpose of this presentation is to provide awareness and education regarding the potential risks associated with post-processing procedures that may lead to tahini contamination or support the growth of *Salmonella* if already present.

Methods: During the Ontario outbreak investigations, local public health units conducted epidemiological and onsite environmental investigations for each outbreak. In addition, the outbreak management team reviewed findings and coordinated additional actions to identify risk and possible mitigating measures. Literature on low-moisture foods and other relevant information on tahini and *Salmonella* were reviewed to further characterize the problem and next steps.

Results: The outbreak investigations identified that tahini had been furthered processed by various food premises. This may result in cross-contamination and/or allowed for growth of *Salmonella* and/or other pathogens, if present. This presentation will highlight the findings and provide information on potential risk factors and control measures. Examples of risk factors that will be discussed include inadequate cold holding practices, dilution, and inclusion of other ingredients which may introduce opportunities for cross contamination. This presentation will also include findings from literature review regarding potential growth and survival characteristics of *Salmonella* in low-moisture food, including tahini.

Significance: This presentation will address the foodborne illness risk with a trending food item. Through lessons learned from multiple field investigations of *Salmonella* and tahini, several key factors that may increase the risk will be discussed. We hope by increasing awareness, we can collectively reduce the potential risk of foodborne illnesses associated with tahini.

Poster

P1-199 Performance and Transcriptome Analysis of *Salmonella enterica* Serovar Enteritidis PT 30 Under Desiccation Stress: Cultured by Lawn and Broth Methods

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Introduction: Lawn-harvest method uses a solid medium (e.g., tryptic soy agar, TSA) to produce bacterial lawns and is widely accepted for culturing microorganisms in microbial studies of low-moisture foods (LMFs, foods with water activity less than 0.85). It appears to produce desiccation-tolerant cells with higher D-values in LMFs; however, little is known about the molecular mechanisms underlying the bacterial performance.

Purpose: We aimed to track the transcriptomic differences between two *Salmonella* cultured groups (lawn-cultured and broth-cultured) in response to desiccation and heat and revealed the molecular mechanisms underlying their different suitability in microbial studies of LMFs.

Methods: Salmonella *enterica* Enteritidis PT 30 (*S.* Enteritidis), the most pertinent pathogen in LMFs, was cultured on TSA and tryptic soy broth (TSB). Cells were harvested and inoculated on filter papers for accessing their heat resistance and survivability at a relative humidity of 32±0.02%. Transcriptome analysis of cultured cells during desiccation (at 24, 72, and 168 h) was conducted in TruSeq PE Cluster Kit v30cB0t0HS (Illumina) by paired-end methods.

Results: Lawn-cultured *S*. Enteritidis cells have stronger survivability and heat tolerance than those from the broth method. More desiccation genes of lawn-cultured cells were significantly up-regulated from growth to desiccation. Different expressed genes were most enriched in Ribosome and Sulfur metabolism pathways in the lawn- and broth-cultured groups, respectively.

Significance: This study supports the usage of the lawn-harvest method in LMF-related microbial studies, and would increase our understanding of differential stress responses of *S*. Enteritidis to environmental stressors, including desiccation and heat treatment.

P1-200 Temporal Changes in Shiga-Toxin Producing *Escherichia coli* (STEC) O121 Transcriptome during Storage in Bleached Flour

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Introduction: Persistence of Shiga-toxin producing *Escherichia coli* (STEC) within low-moisture foods, such as flour, has led to several STEC-related outbreaks associated with consumption of raw or undercooked flour products once considered microbiologically safe. The molecular mechanisms by which STEC can survive in flour are not well understood.

Purpose: This study sought to develop an effective sample treatment and RNA extraction method for polysaccharide-based matrices like flour; and to profile STEC transcriptome changes during storage in flour.

Methods: All-purpose bleached flour was inoculated with STEC O121 at 10^9 CFU/g flour. Flour samples were collected at nine time points within the first 48 hours post inoculation and treated with different centrifugation-filtration schemes. The RNA extraction method was optimized, and, after total RNA extraction of all samples, libraries were prepared using the Illumina Stranded Total RNA Prep kit and sequenced on the Illumina NextSeq2000 platform. The raw reads were quantified using salmon and analyzed with DeSeq2 to identify temporal changes in the transcriptome profile upon flour inoculation and storage.

Results: Cellular loss and residual flour in the filtrates were successfully minimized by electing to utilize a 1 µm pore-size membrane filter following quick centrifugation, which consistently led to isolation of high-quality RNA. Preliminary analysis revealed several genes were differentially expressed in all flour samples relative to a pure culture control. Approximately 1200 genes were expressed significantly differently ($P_{adj} < 0.05$) between culture control and flour inoculation. Expression levels for temporally associated genes, mainly stress response and metabolic genes, had a major spike in the rate of expression change at inoculation. However, the levels appeared to stabilize after five hours of storage at which point few genes were differentially expressed relative to the prior timepoint.

Significance: Transcriptomic profiling of STEC persistence within flour will increase the understanding of STEC survival in low-moisture environments and help develop new mitigation strategies.

P1-201 Influence of Sub-Lethal Food Processing-Related Stresses on the Ultraviolet-C Resistance of *Salmonella enterica* and *Enterococcus faecium* NRRL B-2354 on Raw Whole Almonds

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Introduction: Pre-exposure to sub-lethal stresses can alter the cellular physiology of foodborne pathogens such that they become resistant to subsequent treatments, which poses a potential health hazard to consumers.

Purpose: The purpose of this research was to investigate 1) how sub-lethal food processing-related stresses influenced the ultraviolet-C (UV-C) resistance of *Salmonella enterica* on raw whole almonds (RWAs), and 2) whether *Enterococcus faecium* NRRL B-2354 can serve as a surrogate for *S. enterica*.

Methods: A cocktail of three strains (*S.* Enteritidis PT 30, Newport ATCC 6962, and Typhimurium ATCC 14028), as well as ATCC 14028 wild type and its Δ*rpoS* mutant (IB43), and NRRL B-2354, were exposed to sub-lethal desiccation, oxidation, heat shock, or acid stress. All strains were induced to be resistant to 100 µg/ml rifampin using the gradient plate method. RWAs were inoculated with control (unstressed cells in sterile 0.85% saline) or pre-stressed cells at ~6.0 log CFU/sample unit (ten RWAs) and then subjected to UV-C (500 µW/cm²) for 60 min. Tryptic soy agar containing 100 µg/ml rifampin was used for bacterial enumeration.

Results: The populations of acid-stressed *S. enterica* cocktail were higher (*P*<0.05) than unstressed, desiccation-stressed, oxidation-stressed, and heat-shocked *S. enterica* cocktail during exposure to UV-C. The 60-min treatment led to 2.0-, 1.4-, 2.6-, 3.3-, and 0.9-log reductions for unstressed, desiccation-stressed, oxidation-stressed, heat-shocked, and acid-stressed *S. enterica* cocktail, respectively. IB43 was more sensitive (*P*<0.05) to UV-C than ATCC 14028 regardless of whether they were stressed or not. NRRL B-2354 survived similarly (*P*>0.05) to or better (*P*<0.05) than the *S. enterica* cocktail.

Significance: Our results highlight the cross-protection of acid-stressed *S. enterica* on RWAs against UV-C, in which *rpoS* could play a critical role. This study also demonstrates NRRL B-2354 can serve as a suitable surrogate by providing a margin of safety when validating UV-C treatment for inactivating *S. enterica* on RWAs.

P1-202 Gaseous Chlorine Dioxide Reduced *Salmonella* populations on Almonds While Accelerating Lipid Oxidation during Storage

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Introduction: Almonds, which are rich in "good" lipids and other nutrients, have been implicated in several *Salmonella* outbreaks. Earlier studies have demonstrated that gaseous chlorine dioxide ($gClO_2$) is effective in reducing populations of *Salmonella* on various foods. However, studies on applying $gClO_2$ to inactivate *Salmonella* on almonds are limited and there is no study on the impact of $gClO_2$ on lipid oxidation of almonds.

Purpose: The objective of this present study was to evaluate the effects of $gClO_2$ fumigation on *Salmonella* populations and lipid oxidation of almonds, during post-fumigation storage.

Methods: Dehulled, raw, whole nonpareil raw almonds (*Prunus dulcis*) inoculated with a two-strain cocktail of *Salmonella* Typhimurium (ATCC #53647 and 53648) were treated with $gClO_2$ precursors (0.3 g/g almond) in 4-L glass jars for 2.5 h at 55 °C. Non-treated almonds and those heated at 55 °C (without $gClO_2$) for 2.5 h served as controls. After treatments, *Salmonella* cells were recovered from the almonds and enumerated on XLT-4 agar plates. For analysis of lipid oxidation, non-inoculated almonds were treated similarly and stored at accelerated storage (39°C) for three months. Peroxide values, conjugated diene, free fatty acids, and thiobarbituric acid-reactive substances (TBARS) were measured immediately after treatments, and after one and three months of storage.

Results: Results showed that $gClO_2$ treatment at 55 °C reduced populations of *Salmonella* Typhimurium by 2.91-4.29 log CFU/g, depending on the number of almonds, in the treatment jars. Although $gClO_2$ did not have any significant effect on lipid oxidation of almonds immediately after treatments, lipid oxidation accelerated in $gClO_2$-treated almonds during storage as indicated by significant (*P*<0.05) increases in the amounts of peroxide values, free fatty acids, conjugated dienes and TBARS.

Significance: The study may help the almond industry in deciding on the application of $gClO_2$ in order to improve the safety of almonds.

Poster

P1-203 Non-Thermal Inactivation of *Salmonella* spp. in Selected Low-Moisture Foods during Long Term Storage

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* has been implicated in several recent outbreaks linked to low-moisture foods (LMFs). Methods to reduce or eliminate the survival of *Salmonella* in LMFs are needed.

Purpose: Two antimicrobial compounds were investigated for their ability to inactivate *Salmonella* Cubana and *Salmonella* Muenchen in walnuts, hazelnuts, sunflower seeds, and raw wheat kernels during a 26-week storage period.

Methods: Three biological replicates of wheat kernels, sunflower seeds, hazelnuts and walnuts were inoculated with *S*. Muenchen and *S*. Cubana. Wheat kernels were treated with 2 tempering solutions at 2 concentrations: 5% Lactic acid (2mL, 4mL) and Neo-Temper (2mL and 4mL) diluted in 38mL and 36mL of water respectively and applied at a rate of 40mL per Kg. Neo-Pure solutions prepared as 2%Neo-synergy One, 20%Neo-synergy Two and 78% water were used to treat hazelnuts (40mL/Kg), walnuts (60mL/Kg) and sunflower seeds (80mL/Kg). Samples were then stored for 26 weeks post-inoculation. Samples were enumerated in triplicate at 2-week intervals. Enumeration was performed on TSA, as per FDA protocols for enumeration in almond process validation.

Results: In all of the food matrices, *Salmonella* populations declined significantly in the first week when using the Neo-Temper and Neo-Pure solutions. In wheat kernels, Neo-Temper had a greater log reduction (6.06 log CFU/g) when compared to lactic acid treatments (3.53 log CFU/g) over the 26-week period. Similarly, in walnuts (3.85 log CFU/g), hazelnuts (3.69 log CFU/g) and sunflower seeds (4.21 log CFU/g), Neo-Pure significantly reduced *Salmonella* populations ($P \leq 0.05$) when compared to the control.

Significance: Currently, there are limited approaches to reduce the survival of *Salmonella* and other foodborne pathogens in LMFs. This study indicates the potential of two antimicrobial compounds (Neo-Temper and Neo-Pure) to reduce the survival of *Salmonella* spp., in several LMFs including nuts, seeds and wheat (kernels).

P1-204 Title: Survival of a *Salmonella* Enteritidis Bacteriophage-Insensitive Mutant in Wheat Kernels

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❖ ***Developing Scientist Entrant***

Introduction: Recent studies have indicated that mutations in lipoloysaccharide (LPS) affect the ability of *Salmonella enterica* to survive in low-moisture foods (LMFs).

Purpose: To assess the survival in a LMF and characterize the biochemical profile of a *S*. Enteritidis Bacteriophage Insensitive Mutant (BIM).

Methods: Individual wheat kernel samples were inoculated with *S*. Enteritidis SB7 (wildtype isolate) and SB7 B (which is a BIM with a mutation in gene rfbP, required for O-antigen biosynthesis, and previously shown to reduce *Salmonella* survival in LMFs). Samples were enumerated in triplicate on TSA, for a period of 2 weeks. Additionally, the biochemical profiles of SB7 and SB7 B were assessed through the use of phenotypic microarrays (PMs) (Omnilog). The PMs were incubated in the Omnilog for 2 days and an optical density reading was taken every 15 minutes to generate a metabolic profile of the wild type and BIM. The metabolic profiling was complemented by integrating the PM data with whole genome sequence data from SB7 and SB7 B using Duct-Ape.

Results: In wheat, SB7 B decreased by 0.42 log units compared to SB7, indicating that the rfbP mutation did not affect the survival of the *S*. Enteritidis BIM. The PM experiments indicated major changes in the biochemical profiles of SB7 B, including the ability to metabolize many amino acids, carbohydrates, carboxylic acids and peptides, and the inability to metabolize a number of phosphorous and sulfur compounds. Additionally, SB7 B became more sensitive to many antimicrobial compounds, including Colistin, Cephalothin, Cetylpyridinium Chloride and Oxytetracycline, and more resistant to Benzethonium Chloride, Tetracycline and the biofilm inhibitor Thiosalicylate.

Significance: Comparing the phenotypic and genotypic characteristics of *Salmonella* wildtype and BIM isolates can lead to the identification of specific genes and mutations responsible for altered survival. This information can be used to develop control strategies to reduce bacterial survival in LMFs.

P1-205 Inactivation of Desiccation-Resistant *Salmonella* on Apple Slices Following Treatment with Epsilon-Polylysine, Sodium Bisulfate or Peracetic Acid and Dehydration

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Introduction: *Salmonella* is capable of surviving dehydration processes of various foods, including dried fruit. Dried fruit, including apple slices, have been the subject of recalls due to contamination with *Salmonella*.

Purpose: A study was conducted to determine the survival of *Salmonella* on apple slices following antimicrobial immersion treatments (epsilon-polylysine (EP), sodium bisulfate (sodium acid sulfate, SAS) or peracetic acid (PAA)) and subsequent dehydration. Previously published studies demonstrated that 0.50% SAS treatment, prior to dehydration, inactivated >5.0 log CFU/slice of *Salmonella* on Gala apples.

Methods: Gala apples were aseptically cored and sliced into 0.4 mm rings and inoculated with a five-strain composite of desiccation-resistant *Salmonella* at a population of 8.28 log CFU/slice. Slices were then immersed for 2 min in varying concentrations of antimicrobial solutions, including EP (0.00, 0.005, 0.02, 0.05, and 0.10%) SAS (0.00, 0.05, 0.10, 0.20, 0.30, and 0.40%), PAA (0 or 18 ppm), or 18 ppm PAA + the five concentrations of EP, for 2 min and dehydrated at 60°C for 5 hours. Experiments were performed in triplicate, samples were analyzed, and *Salmonella* populations were determined.

Results: Untreated control treatments (washed in sterile water and dehydrated) resulted in reductions of 2.68 log CFU/slice *Salmonella* ($p < 0.05$). Inactivation following EP and SAS treatments increased with increasing concentrations with maximum reductions of 3.87 and 5.52 log at 0.10 and 0.40%, respectively. Based on preliminary studies, higher concentrations of EP did not result in greater inactivation than occurred with 0.1% concentration. Treatment with PAA reduced populations of *Salmonella* by 4.38 log and combining PAA with up to 0.1% EP elicited no greater log reductions.

Significance: These results may be applicable to the food industry as a means for increasing the inactivation of Salmonella during the dehydration of apple slices.

Poster

P1-206 Patented Organic Peracetic Acid and Hydrogen Peroxide-Based Sanitizing Solution Achieves Minimum 4-Log CFU/g Reduction of *Salmonella* Surrogate *Pediococcus acidilactici* ATCC 8042 on Almonds at an Industrial Scale

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Introduction: Sporadic contamination of almonds by *Salmonella* has caused recalls and outbreaks, regulating a pasteurization step for almonds. The efficacy of a commercial pathogen control technology involving application of a patented organic acid and hydrogen peroxide solution followed by drying was evaluated at an industrial scale.

Purpose: To 1) establish laboratory scale parameters for a minimum 4 log CFU/g reduction of *Salmonella* 2) identify an appropriate surrogate for *Salmonella*, 3) evaluate reduction of the surrogate at an industrial scale, and 3) assess the sensory attributes.

Methods: Almond batches inoculated with different *Salmonella* serovars (n=10), *E. faecium* NRRL B-2345 or *P. acidilactici* ATCC 8042 at >1.0×10^7 CFU/g, were treated (100 mL/kg), dried (104.4°C for 7 min), and cooled (20°C for 3 minutes). Sub-samples (5x50 g) were enumerated before and after treatment using the FDA Bacteriological Analytical Manual enumeration procedure. Three industrial scale validations were completed. For each, 28 kg of dyed almonds were inoculated with surrogate *P. acidilactici* ATCC 8042, mixed with fresh almonds (~1,500 kg), treated in an applicator (100 mL/kg), and conveyed into a fluidized bed dryer (113°C for 7:19 min). Inoculated dyed almonds were sorted for enumeration (~35 × 45 g sub-samples). Recovery was compared to inoculated, untreated samples (10 × 45 g). Fresh almonds treated under the same parameters were blindly assessed for the five sensory attributes (5-point hedonic scale, 10 participants).

Results: Laboratory scale results demonstrated a >4-log reduction of *Salmonella* and identified *P. Acidilactici* ATCC 8042 as an appropriate surrogate for industrial scale validation. A minimum 4.37 log CFU/g reduction of the surrogate *P. acidilactici* ATCC 8042 was achieved with the Neo-Pure Food Safety System at the industrial scale (n=3), with sensory attributes unchanged.

Significance: The industrial scale system is a suitable intervention method complying with USDA regulations for the pasteurization of almonds, while maintaining sensory attributes.

P1-207 Control of *Salmonella enterica* in Soft Wheat Berries by Tempering Solutions Containing Lactic Acid

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Introduction: Food safety outbreaks have occurred in low water activity products, including flour. Pathogen population in flour can be reduced via application of antimicrobials in the tempering solution. Lactic acid has demonstrated efficacy at reducing microbial counts in hard wheat berries when applied during tempering, but efficacy during the shorter tempering periods with lesser water absorption in soft wheat warrants investigation.

Purpose: Evaluate *Salmonella* reductions in soft wheat following tempering in lactic acid solutions.

Methods: Soft wheat berries were dried (8.50% moisture) to compensate for the addition of inoculum and autoclaved to achieve sterility. Wheat (200 g) was inoculated at 6 log CFU/g with a 5-strain *Salmonella enterica* cocktail. Post-24 h attachment period (20°C), wheat was tempered 6 h at 20°C with a consistent volume (3.49 mL) but increasing concentrations (0 [water control], 20, 40, 60% w/v) of lactic acid (PURAC® FCC88) in water to achieve ca. 14.5% moisture. Post-tempering, three 50 g samples were serially diluted in Butterfield's buffer and spread-plated on xylose lysine tergitol-4 agar.

Results: Initial *Salmonella* population in pre-treated inoculated wheat was 5.27 log CFU/g. There was no difference (p>0.05) in *Salmonella* count (4.73 log CFU/g) in water control versus pre-treatment. There was also no difference (p>0.05) in wheat treated with 20% lactic acid (4.55 log CFU/g) compared to water control; however, 20% lactic acid had significantly lower (p<0.05) counts than pre-treatment. Application of 40% and 60% lactic acid resulted in populations (3.77 log CFU/g and 2.72 log CFU/g, respectively) significantly (p<0.05) lower than pre-treatment and water control.

Significance: Lactic acid efficacy has been previously well established in hard wheat tempering where temper times and moisture addition is appreciably greater than with soft wheat tempering. The application of lactic acid during tempering can reduce *Salmonella* counts in soft wheat and may provide microbial safety in downstream products.

P1-208 Antimicrobial Washes on In-Shell Pecans Inoculated with Shiga Toxin-Producing *Escherichia coli*

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Introduction: Presently, small-scale pecan growers are exempt from the Food Modernization Act (FSMA), but would benefit from implementing preventive controls, such as antimicrobial washes to address contamination risks. Shiga toxin-producing *E. coli* can contaminate pecans through soil in orchards, which could impact the efficacy of washes.

Purpose: This research determined the efficacy of antimicrobial washes in reducing STEC on in-shell pecans and the effects of soil on the efficacy of the wash.

Methods: Pecans were directly spray-inoculated with STEC (O157:H7, O157:NM, O121, and O26) and indirectly by coating with inoculated soil. After 24-hr storage at ambient conditions, pecans were washed with treatments of 2% lactic acid, 1000 ppm sodium hypochlorite, hot water (85 ± 2°C), and ambient water (control) for 2, 5, and 10 min. STEC were enumerated from pecans using *E. coli*/Coliform Petrifilm™. Log reductions from the washes were calculated and analyzed using JMP Pro with ANOVA and pairwise comparisons.

Results: Five-minute treatments were as effective in reducing STEC as 10-min treatment whether soil was present or not. The STEC reductions from a 10min ambient control wash on direct and soil-inoculated pecans was 1.4±0.8 and 0.3±0.9 log CFU/g, respectively. The reductions for direct and soil-inoculated pecans treated with LA for 10 min were 3.6±0.4 and 1.0±0.6 log CFU/g, respectively. The reduction for the 10min lactic acid treatment was 3.6±0.4 and 1.0±0.6 log CFU/g on direct and soil-inoculated pecans, respectively. Hot water was the most effective treatment on soil-inoculated pecans (>5 log CFU/g; p<0.05). No significant differences were found between ambient control, LA, and sodium hypochlorite treatments on soil-inoculated pecans (p<0.05). The presence of soil reduces the antimicrobial efficacy of the lactic acid and sodium hypochlorite treatments.

Significance: Soil and debris can reduce sanitizer efficacy on pecans. Future research will identify cost-effective interventions for preventative controls in post-harvest processing for small pecan growers.

P1-209 Comparison of Thermal Inactivation of *Enterococcus faecium* NRRL B-2354, *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* in Sweet Potato and Coconuts at Three Moisture Levels

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Introduction: Low moisture foods such as dried vegetables can be contaminated with salmonella and other pathogens of concern. The proper mitigation step must be validated under the FSMA Final Rule for Preventive Controls for Human Food.

Purpose: This study investigated the thermal death time characteristics of *E. coli, L. monocytogenes*, *Salmonella* and *E. faecium* in sweet potatoes at three moisture levels.

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Methods: Samples were inoculated with *E. faecium,* a cocktail of *E. coli, L. monocytogenes*, or *Salmonella* at approximately 7 log CFU/g and acclimated to 25°C for 20 min. Inoculated samples were dispensed into pouches, vacuum sealed, exposed to 60 to 85°C in water bath and pulled at predetermined intervals. Surviving organisms were counted, averaged, then transformed to log CFU/g.

Results: *E. faecium* was significantly more heat resistant in at all moisture levels compared to *E. coli*, *L. monocytogenes* and *Salmonella. E. faecium* was two times (>2) more heat resistant than the test pathogens at lower moisture levels (i.e. 5%) This ratio was larger for higher moisture levels. For example, the *E. faecium* to *Salmonella* ratio was approximately 4.6 at 16.56% moisture and 70°C. *Salmonella* D values in sweet potato at 70, 75, and 80°C for 5.03% moisture (aw= 0.3) were 13.55, 6.65 and 2.94 min, respectively, while the D values at 65, 70 and 75°C for 8.01% moisture (aw= 0.46) were 20.41, 6.99 and 5.14 min, respectively, and for 16.56% moisture (aw= 0.68) were 11.5, 5.01, and 2.0 min, respectively.

Significance: *Salmonella* resistance increased as product moisture level decreased. Thermal resistance data showed that *E. faecium* would be a suitable surrogate for in-plant validation studies of sweet potatoes during drying. This data can be used as a scientific basis for thermal validation of fruits and vegetables with similar moisture.

P1-210 Effect of Temperature and Airflow on Inactivation of Enterococcus faecium NRRL B-2354 in Apple Cubes during Hot Air Drying

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❖ ***Developing Scientist Entrant***

Introduction: Higher airflow can accelerate the drying process, but its effect on microbial lethality is unknown.

Purpose: To evaluate the effects of high-temperature, low-airflow (HTLA) and low-temperature, high-airflow (LTHA) conditions on Enterococcus faecium and water activity (a_w) of apple cubes throughout drying.

Methods: E. faecium was grown on tryptic soy agar with 0.6% yeast extract (TSAYE). The culture was harvested in buffered peptone water and inoculated onto cored, peeled Gala apple cubes (~6.4 mm) at 8.66±0.15 log CFU/sample (n=4 apple cubes). Apple cubes were dried using a vertically directed heat source at a fixed bed depth (12.7 cm) at 135°C, 25% airflow setting (HTLA) and 104°C, 50% airflow setting (LTHA). Triplicate samples were taken from the top layer of the bed to measure a_w and microbial population throughout drying (n=6). E. faecium was enumerated on eTSAYE (TSAYE with 0.05% ammonium iron citrate and 0.025% esculin hydrate, 97%).

Results: The a_w after 100 min was similar for HTLA and LTHA samples, 0.64±0.26 and 0.49±0.20, respectively (p>0.05). Microbial reductions after drying 100 min were 5.17±2.11 and 4.16±2.20 log CFU/sample for HTLA and LTHA, respectively. No significant difference in E. faecium reduction was observed between the drying conditions after 100 min (p=0.15). A greater than 5-log reduction was achieved in all samples after 110 min and 120 min for HTLA and LTHA conditions, respectively. However, linear regression models fitted to the microbial reduction and the a_w of the dried apple cubes showed significantly less (p<0.05) microbial reduction overall during LTHA than HTLA as the a_w decreased. HTLA conditions produced more browned dried apple cubes than LTHA conditions.

Significance: A 5-log reduction was not achieved before a_w 0.6 was surpassed under LTHA conditions. Longer drying time is needed for LTHA to achieve a 5-log reduction. An intermediate condition may be needed to maintain quality while providing an appropriate microbial inactivation.

P1-211 Determination of the Thermal Inactivation Kinetics of *Salmonella* and a Surrogate in Milk Powder as Impacted by Water Activity and Food Matrix

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Introduction: Milk powders may be considered ready-to-eat and have caused multiple foodborne illness outbreaks, such as powdered infant formula. Presently, no kill-step exists in milk powder production post-spray drying. Contamination and subsequent pathogen survival in the powder could result in outbreaks/recalls.

Purpose: The objective of this study was to determine the thermal inactivation kinetics of *Salmonella* and a surrogate in milk powders with low water activity (a_w) to then evaluate the suitability of the chosen surrogate.

Methods: Non-fat dry milk (NFDM) and Milk Protein Concentrate (85%) were inoculated with *Salmonella* spp. and *Enterococcus faecium* NRRL B-2354 (ca. 9.0 log CFU/g) and equilibrated to a_w 0.10, 0.20, or 0.30 ± 0.02 for five days. Microbial thermal inactivation was determined by enumerated the bacteria from milk powders that were processed in thermal-death-time disks submerged in a water bath (75°C, 80°C, or 85°C). Experiments were replicated at least three times to attain D- and z-values.

Results: D-values for both milk powders increased with the decrease of a_w. For *Salmonella* in NFDM at a_w 0.3, D-values were as follows: $D_{75°C}$=13.3±5.4 min, $D_{80°C}$=7.9±0.98 min, $D_{85°C}$=3.7±0.8 min. For *Salmonella* in NFDM at a_w 0.2, *D*-values were as follows: $D_{75°C}$=16.2±1.3 min, $D_{80°C}$=7.1±1.8 min, $D_{85°C}$=3.2±0.5 min. For *Salmonella* in NFDM at a_w 0.1, *D*-values were as follows: $D_{75°C}$=76.5±12.6 min, $D_{80°C}$=30.4±0.8 min, $D_{85°C}$=17.9±2.1 min. D-values for *E. faecium* were higher on average than *Salmonella*, and D-values for each microorganism were significantly higher in MPC-85 than in NFDM.

Significance: Overall, *E. faecium* exhibited greater thermal resistance than *Salmonella* in most experiments, lending to its potential suitability as a surrogate for *Salmonella* in thermally treated NFDM and MPC. The difference between D-values associated with the powders highlight the protective effects some food matrices have for microorganisms and that thermal processes should be designed specific to each food, rather than water activity alone.

P1-212 Acidic Tempering and Heat Treatment-Based Hurdle Approach to Reduce *Salmonella* Load in Wheat and Its Impact on Wheat Flour Quality

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❖ ***Developing Scientist Entrant***

Introduction: The number of foodborne illness outbreaks and recalls associated with wheat flour and wheat-based products contaminated with enteric pathogens such as *Salmonella* has increased in the past decade. This shows the need for effective antimicrobial interventions to be incorporated in the milling industry.

Purpose: To investigate the survival of *Salmonella* in wheat after acidic water tempering coupled with heat treatment.

Methods: This research sought to determine survival of four different *Salmonella enterica* serovars on wheat during tempering (hydration). Hard red spring (HRS) wheat kernels were inoculated with *Salmonella* (cocktail ~7 log CFU/g) and were dried for 24h at ambient temperature. Following which, wheat kernels were tempered to 17% moisture with sodium bi sulfite (SBS), lactic acid (LA) and/or citric acid (CA) solution (10 and 15% w/v concentration) and were held at 25°C and/or 55°C. Wheat kernels tempered with sterile water was taken as a control.

Results: The study's findings demonstrated that, after 24h of treatment, wheat tempered with acidic water (15% w/v) reduced the *Salmonella* load by at least 3 log CFU/g for all the tested acids. Heat treatment (55°C) alone significantly reduced the *Salmonella* load while acidic water tempering coupled with heat treatment (55°C) resulted in greater reduction with complete inhibition achieved at 12h for LA and 18h for both SBS and CA. Furthermore, the study also demonstrated the production of reactive oxygen species (ROS) by virtue of which, protein and sugar leaked from the bacterial cell leading to their lysis. Additionally, Tempering HRS wheat at 15% concentration did not significantly alter the baking (volume, texture, and crumb structure) or physicochemical properties (rheology, composition) of the treated wheat flour compared to the control.

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Significance: Acidic water tempering, as opposed to the traditional water tempering method, may result in milled products with improved microbiological quality.

P1-213 Effect of Inoculation Level, Tempering Treatments, and Time on the Distribution of *E. coli* in Hard Wheat Milling Fractions

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Developing Scientist Entrant

Introduction: Pathogen contamination in wheat flour milling have become a concern; this is because of the increase in recalls and food borne illness outbreaks involving wheat flours.

Purpose: The objective of this study is to evaluate the effect of different tempering conditions (time, tempering treatment, and inoculation level) on the distribution of *E. coli* on the mill fractions obtained during lab-scale roller milling.

Methods: Hard red winter wheat grains (HRW) were obtained and pre-dried overnight (50°C, 12 h) to prevent excess moisture gain during the experiments. The prepared wheat grains (500g/ run; 10 runs) were inoculated with non-pathogenic *E. coli* (ATCC 1427, 1429, 1430, and 1431) at 3 and 6 log CFU/g. Inoculated grains were then tempered to 16% moisture under different tempering times (8 and 12 h) and tempering solutions (0, 5, 10% acid). Tempered grains are then milled using a lab-scale roller mill and mill fractions (flour, bran, and shorts) were collected and analyzed for their *E. coli* load.

Results: The use of the acid tempering solutions (5 and 10%) reduced ($P < 0.05$) the *E. coli* load recovered in the mill fractions at both inoculation levels used by 1 to 2 log CFU/g. Furthermore, their use also lowered the rate of increase in the *E. coli* load of the flour fractions during the mill run. Increased tempering time also reduced the *E. coli* load recovered in the mill fractions.

Significance: The results from this study will help serve as a basis for improving wheat flour food safety against pathogen contamination through tempering methods.

P1-214 Predicting the Impact of Tempering Treatments on the *E. coli* Load Reduction during Tempering and Its Subsequent Effects on Flour Quality

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Introduction: Various interventions applied during the tempering step have been developed to help mitigate pathogen contamination in wheat flours. Varying effects have been observed which could allow models to be developed.

Purpose: The objective of this study is to model the effects of different tempering treatments and tempering conditions to predict the reduction in *E. coli* load in the wheat and its subsequent impacts on wheat flour milling and baking quality.

Methods: Existing data on the various tempering treatments will be used including reductions observed during acidic water tempering (sodium bisulfate, lactic acid, and plasma water), mild heating, and bacteriophage tempering. Their effects on wheat flour milling and baking quality will also be included. Models will then be fitted to the data set with the treatment doses and tempering conditions as inputs. The *E. coli* load reductions and flour quality parameters will then be the output of the model. Model fit will be assessed by checking model parameters including R^2, standard error, and AIC values.

Results: Model fitting revealed that the treatment dose were significant in predicting the *E. coli* load reduction and flour quality changes. Acceptable model fit parameters including R^2 (0.6- 0.7), standard error (10-15%), and lower AIC values were also given for the model selected.

Significance: The results from this study could be used as a basis in filtering potential interventions applied during wheat milling which could help reduce costs of experiments.

P1-215 Reduction of *Salmonella* Loads through a Heat Treatment on Different Flatbread and Pancake Mixes

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Developing Scientist Entrant

Introduction: Flatbread and pancake mixes with ingredients as organic banana, organic cassava, quinoa, and sweet potato are still considered as knew. It is necessary to perform analyzes on them to know if there are *Salmonella* counts and if a certain ingredient attach the pathogen more when inoculating the batter. After cooking, there should be no *Salmonella* counts on the final products.

Purpose: Determine the *Salmonella* level of reduction after the cooking process.

Methods: Studies for flatbread and pancake mix followed the same process. Flatbread treatments: Organic green banana (1f) and Organic amazonian cassava (2f). Pancake treatments: Organic green and yellow banana (1p), Quinoa and oats with cinnamon (2p), Quinoa and oats with chocolate chips (3p), and Green banana and sweet potato (4p). The *Salmonella* cocktail (8 LogCFU/mL) consisted of three *Salmonella* strains: Typhimurium, Newport, and Enteritidis. Batter was inoculate and negative controls were included. After cooking, the final product was weight (3 samples/treatment). The *Salmonella* enumeration on the batter followed the drop dilution method on XLD (triplicate). On the final product, the *Salmonella* enumeration was done by spread plating on XLD. Statistical analysis was perform on SAS between treatments and between sampling points.

Results: Negative controls were zero. The mean separations of attachment on pancake (P-valor = 0.4455) and flatbread (P-valor = 0.5352) treatments did not show differences. In average, the pancake attachment was 5.98 LogCFU/g and the flatbread one was 5.38 LogCFU/g. Nevertheless, the difference between attachment and reduction was obvious, with p-values of <0.001 for both studies. Even though, there were no differences between treatments after cooked, some treatments (2f, 2p, 3p, and 4p) showed low counts of *Salmonella* (<1CFU/g).

Significance: This study helps to make sure that food is safe for the consumer. As well, it shows how uncommon ingredients attach pathogens and how their load reduces because of the heat treatment.

P1-216 Lethality of *Salmonella* spp. Inoculated Oats in Multiple Granola Formulations during Oven Baking Compared to Thermal Lethality Calculator

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Introduction: Process validation aimed at addressing *Salmonella* spp. in low moisture foods, like granola, is an essential part of any food safety plan. A conservative thermal lethality calculator (TLC) was used to estimate the log reduction of *Salmonella* spp. in baked granola based on temperature and water activity testing. However, because of the low water activity (a_w) inherent with the granola product, the TLC resulted in a lower *Salmonella* lethality than the target five-log reduction.

Purpose: The purpose of this study was to determine the lethality of *Salmonella* spp. inoculated oats flakes mixed into three granola formulations after oven baking and compare to the conservative TLC results.

Methods: Oats were initially mist inoculated with *Salmonella* spp. and dried back to pre-inoculation a_w. *Salmonella* spp. inoculated oats were weighed out and mixed into au natural, chocolate, and original granola formulations. Three trays of each granola formulation (8800 g) were baked at 275°F for 170

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minutes per the processing at the facility. *Salmonella* spp. populations in pre- and post-baking granola samples (25 g) were enumerated on tryptic soy agar with a xylose lysine deoxycholate agar overlay.

Results: TLC estimated the log reduction as 3.73 ± 1.41 log (CFU/g). *Salmonella* spp-inoculated oats mixed into granola showed an average 6.87±0.27, 6.81±0.92, and 7.12±0.54 log (CFU/g) reduction when baked at 275°F over 170 min for au natural, chocolate, and original granola formulations, respectively. The a_w of post-baked granola was between 0.423 and 0.552. The a_w of post-baked and dried granola was between 0.163 and 0.317.

Significance: Reduction of *Salmonella* spp. during oven baking of granola is not consistent with TLC when using final post-baked and dried granola a_w. It is important to consider moisture loss from drying after thermal processing as it may affect the TLC results.

P1-217 >4 Log CFU/g Reduction in *Salmonella* surrogate *Enterococcus Faecium* nrrl B-2354 and >1 Log CFU/g Reduction in Aerobic Colony Counts (ACC) Achieved on Dehydrated Onion Flakes Using Industrial Scale Pasteurization System

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Introduction: In the last three years, North America has seen 44 recalls of onions associated with *Salmonella* contamination which caused 429 reported hospitalizations. Previous work with a pilot-scale pathogen control technology involving application of a proprietary sanitizing solution to dehydrated onion flakes, followed by drying, achieved a >4 log CFU/g reduction in *Enterococcus faecium* NRRL B-2354, and a >1 log CFU/g reduction in Aerobic Colony Counts (ACC), warranting scale up to an industrial system.

Purpose: To investigate the efficacy of an industrial-scale pathogen control technology against the *Salmonella* surrogate *E. faecium* and ACC levels on dehydrated onion flakes.

Methods: Inoculated onion flakes (1361 kg, 60 mL/kg *E. faecium* culture) were treated with the sanitizing solution (75 mL/kg) in an industrial scale applicator, followed by drying (132 °C for 2:55 min) in a fluidized-bed dryer (3 MT/hr). In a separate run, un-inoculated flakes (1134 kg) were treated (60 mL/kg) and dried (127 °C for 2:29 min) to evaluate ACC levels. Samples were collected before and after process every 2 minutes for *E. faecium* enumeration (22.5 g, n=14 on *Enterococcus*-selective agar plates) and every 3 minutes for ACC enumeration (22.5 g, n=7 on 3M™ Petrifilm™ AC plates). Additionally, percent moisture content (MC) and water activity (a_w) of sub-samples were measured.

Results: An average 4.61 ± 0.58 and 1.15 ± 0.06 log CFU/g reduction was achieved for *E. faecium* and ACC, respectively. Average MC values for inoculated untreated and treated flakes were 7.49% and 6.30%, respectively, with a_w readings of 0.44 and 0.29, respectively. Average MC values for un-inoculated untreated and treated flakes were 6.57% and 5.79%, respectively, with a_w readings of 0.29 and 0.20, respectively.

Significance: The industrial-scale system is a suitable pathogen control step on dehydrated onion flakes in reduction of a *Salmonella* surrogate and ACC levels.

P1-218 >5 Log CFU/g Reduction in *Salmonella* Surrogate *Enterococcus faecium* NRRL B-2354 and >1 Log CFU/g Reduction in Aerobic Colony Counts (ACC) Achieved on Dehydrated Garlic Flakes Using Pilot Scale Pasteurization System

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Introduction: Current dehydration methods for garlic act as a partial pathogen control mechanism that typically necessitates >3 hours, despite which, surveys conducted by the FDA have found *Salmonella* in retail samples across the USA. Hence arises the need for a quicker yet comprehensive, and novel pasteurization method for dried garlic flakes.

Purpose: To investigate the efficacy of a pathogen control technology involving application of a proprietary sanitizing solution, followed by drying, against the *Salmonella* surrogate *Enterococcus faecium* NRRL B-2354 and Aerobic Colony Counts (ACC) levels on dehydrated garlic flakes, at pilot-scale.

Methods: Inoculated dehydrated garlic flakes (60 kg, 30 mL/kg *E. faecium* culture) were treated with the sanitizing solution (100 mL/kg) in a pilot-scale applicator, followed by drying (138 °C for 2:13 min) in a fluidized-bed dryer (3 kg/min; scale-up equates to 3 MT/hr). In a separate trial, un-inoculated flakes (60 kg) were treated (100 mL/kg) and dried (138 °C for 2:06 min) to evaluate ACC levels. Samples were collected before and after processing every 2 minutes for *E. faecium* enumeration (45 g, n=10 on *Enterococcus*-selective agar plates) and, every 2 minutes for ACC enumeration (45 g, n=9 on 3M™ Petrifilm™ AC plates). Additionally, sub-samples were analyzed for percent moisture content (MC).

Results: An average 5.84 ± 0.35 and 1.68 ± 0.21 log CFU/g reduction was achieved for *E. faecium* and ACC, respectively. Average MC values for inoculated untreated and treated flakes were 10.80% and 7.79%, respectively. Average MC values for un-inoculated untreated and treated flakes were 9.95% and 7.72%, respectively.

Significance: The pilot-scale pasteurization technology showcases a suitable pathogen control step on dehydrated garlic flakes in reduction of a *Salmonella* surrogate and ACC levels. This warrants a similar study on industrial scale.

P1-219 Steam Inactivation of *Listeria innocua* and *Enterococcus faecium* Nrrl 2354 in Almond Kernels as Impacted by Water Activity

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❖ ***Developing Scientist Entrant***

Introduction: Raw almonds have been implicated in *Salmonella* outbreaks and multiple recalls associated with *Listeria monocytogenes*. Steam is approved for microbial decontamination of almonds. However, limited information is available on the impact of water activities (a_w) on the effectiveness of steam in microbial inactivation.

Purpose: This study evaluated the effectiveness of steam treatments at 100 - 135 °C against *L. innocua* and *Enterococcus faecium* NRRL 2354 in almond kernels and the impacts of a_w on their thermal resistance in almond kernels during the steam treatments.

Methods: Almond kernels inoculated with ~ 8 log_{10} CFU/g *L. innocua* or *E. faecium* were equilibrated to a_w 0.25 and a_w 0.45, and then were subjected to steam treatments at 100 - 135 °C, respectively.

Results: The resistances of *L. innocua* and *E. faecium* were inversely correlated with the a_w of almonds and steam temperatures. *D*-values of *L. innocua* were ~ 3 - 17 sec vs. 2 - 12 sec for almonds a_w 0.25 vs. a_w 0.45 during steam treatments at 100 - 130 °C, respectively. *E. faecium* had higher tolerances to steam treatments than *L. innocua* in almonds; the *D*-values of *E. faecium* in almonds a_w 0.25 vs. a_w 0.45 were ~ 4 - 22 sec vs. 2 - 15 sec for steam treatments at 100 - 135 °C, respectively. The ***z***-values of *L. innocua* were not impacted by the a_w of almonds, while the ***z***-values of *E. faecium* in almonds a_w 0.25 was higher ($P < 0.05$) than that in almonds a_w 0.45. *E. faecium* exhibited less sensitivity to temperature change in steam treatments than *L. innocua*.

Significance: Data collected from this study suggested that steam treatments are effective strategies for mitigating microbial contamination in almond kernels.

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P1-220 Effect of Different Particle Sizes on Iso-Thermal Water Activity and Microbial Heat Resistance in Roasted Peanut

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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 at temperatures ($a_{w,T}$) associated with the controlled heat treatment of the pathogens. Fats and oils have their own unique effect on water activity, but iso-thermal water activity measurements have not been performed for food samples with different particle sizes to quantify the effect of available free oil on $a_{w,T}$.

Purpose: Clarify the effect of different fat exposure levels (by grinding roasted peanut kernels) on the isothermal water activity of food samples and bacterial heat resistance.

Methods: Roasted peanut kernels were selected as a typical high-fat-low-moisture food ($a_{w,25°C}$ < 0.85, fat content ≈ 49%) and were grinded and seived at different degrees (peanut kernel 2-4 mm, crushed peanuts 0.85-2 mm, peanut flour 0.43-0.85 mm, peanut powder 0.43 mm, peanut spread <0.43 mm). The a_w of samples with these samples was measured by using a modified thermal cell and relative humidity and temperature sensors over a temperature range of 25 to 75°C. Bacterial heat resistance of *Salmonella* in selected samples was quantified at controlled moisture contents in thermal-death-time cells.

Results: For test samples with fixed moisture content, the $a_{w,T}$ values usually increased with increasing temperature within a certain range ($a_{w,25°C}$ 0.1 - 0.4), and $a_{w,T}$ values stayed stable for each particle size sample when $a_{w,25°C}$ ≥0.4. The Salmonella heat resistance parameters also vary with peanuts' particle sizes, indicating that different process control is needed for low-moisture foods with different structures.

Significance: We reported the influence of temperature and particle sizes on the iso-thermal water activity for low-moisture high-fat would support the (precise) microbial control of pathogens in LMFs.

P1-221 Thermal Inactivation of *Salmonella* and *Enterococcus faecium* in Raw Peanuts

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Introduction: Foodborne illness due to *Salmonella* and control of it in low-moisture foods, including nuts, is a significant concern for the food industry. Nuts are associated with a food safety risk for *Salmonella* due to their inherent properties as an agricultural product, and due to the techniques used to process them.

Purpose: This study aimed to determine the effects of thermal processing on *Salmonella* lethality as well as its surrogate *Enterococcus faecium* in peanuts.

Methods: Raw peanuts were inoculated with *Salmonella* Enteritidis phage type 30 or *Enterococcus faecium* NRRL B-2354 at ~10^8 CFU/g. Inoculated peanut samples were heated in a laboratory-scale air fryer roaster oven under various conditions. Population of *Salmonella* and *E. faecium* was determined by plating serial dilutions onto Trypticase Soy agar and incubated at 35°C for 24 h.

Results: A five-log inactivation of *Salmonella or E. faecium* was achieved by thermal roasting. For example, a dry roasting of 157°C for 15 min reduced *Salmonella* and *E. faecium* by 7.0 and 6.2 log CFU/g, respectively. *E. faecium* might be a suitable surrogate of *Salmonella* in peanut roasting studies.

Significance: This study validated that dry roasting achieved five-log inactivation of *Salmonella* in raw peanuts and provided scientific evidence for thermal process conditions used by the food industry.

P1-222 Enhanced Heat Resistance of Freeze-Dried *Enterococcus faecium* NRRL B-2354 as Valid *Salmonella* Surrogate in Low-Moisture Foods

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Introduction: In microbial studies of low-moisture foods (LMFs, water activity less than 0.85), freeze-dried bacteria benefit us to inoculate LMFs without introducing extra water nor altering food physiochemical properties in laboratories and pilot plants. However, the freeze-drying process would bring unavoidable damage to bacterial cells and results in less resistant inoculum that are unlikely to be qualified in microbial studies.

Purpose: Enhancement of heat resistance of *Enterococcus faecium* NRRL B-2354, production of lyophilized bacterial powder, and most dried inoculum substitution for *Salmonella enterica* Enteritidis *PT 30* in LMFs for heat resistance studies.

***Methods*:** Herein, we enhanced bacterial heat resistance by subjecting the cells to mild heat (42 to 50 °C) to counteract the reduced heat resistance and survivability of freeze-dried bacteria. *Enterococcus faecium* NRRL B-2354 (*E. faecium*), a *Salmonella* surrogate in LMFs, was used as the target microorganism because it was widely accepted in microbial validation of thermal pasteurizing LMFs. Three types of LMFs (peanut powder, protein powder, and onion powder) were used as LMF models to validate the freeze-dried *E. faecium* in comparison with *Salmonella enterica* Enteritidis PT 30 (*S.* Enteritidis).

***Results*:** The heat resistance (D_{65} value) of *E. faecium* increased at all treatments and peaked (+ 31.48±0.13%) at temperature time combinations of 45°C/60 min and 50°C/5 min. Freeze-drying process only reduced the D_{65} values of *E. faecium* by 18.77% and disqualified it as a *Salmonella* surrogate because *S.* Enteritidis had higher D_{65} values. Survivability of freeze-dried inoculum and its heat resistance retained well within 50 d storage. Freeze-dried *E. faecium* prepared in this study brought equal or higher heat resistance ($D_{85°C}$ or $D_{75°C}$) than *S.* Enteritidis in tested LMFs models.

***Significance*:** The freeze-dried *E. faecium* with enhanced heat resistance appears to be a suitable *Salmonella* surrogate for dry-inoculating LMFs. Our protocol also enables industry-scale production of freeze-dried inoculum by broth-cultivation method.

P1-223 Spice Decontamination Using Microwave and Radiofrequency Technologies

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Introduction: Foods with low moisture content such as powders are often contaminated with bacteria and spores.

Purpose: Microwave and radio frequency were carried out on a spice that was put into 2.5 kg plastic bags, to evaluate the effectiveness of these technologies on the heat treatment of products directly in the packaging.

Methods: The product had initial water content of 7% (dry basis), water activity of 0.350 and initial microbial load (TPC) of 6.30 log CFU/g. A microwave equipment working at 2450 MHz (LABOTRON FL12000, Sairem, France), and a radio frequency machine operating at 27.12 MHz (TRF 08, Sairem, France) with electrode height of 90 mm, were employed for the tests. The incident power was of 3 kW and the temperature inside the bags were controlled by an optic fiber.

Results: With microwave, the temperature at the center of the product achieved 105 °C in 240 s. The sample's temperature was held for 20 min, and at the end of the process a log reduction of 2.70 was obtained. Using radio frequency, the temperature of the powder reached 120 °C at 325 s. This temperature was maintained for 20 min obtaining a log reduction of 6.27 for the treated product. When a surrogate (*Enterococcus faecium*) with a total of 8.40 log CFU/g was put inside the bag and treated by microwave, the reduction of the TPC was of 0.9 and the reduction of the surrogate was of 6. The moisture content and color of samples did not change significantly with the heat treatments.

Significance: These results evidence the potential of microwave and radio frequency on treating low moisture foods even inside their packaging, promoting fast heating and being capable of eliminating microorganisms without major physical modifications on the product.

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P1-224 Effect of Different Storage Conditions and Brewing Methods on the Survival of *Salmonella*

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Introduction: *Salmonella* is the leading causes of bacterial gastroenteritis. Dried teas are traditionally believed to have a low risk of causing salmonellosis. However, in recent years, *Salmonella* has implicated numerous recalls of herbs and teas. Entering into a viable but non-culturable (VBNC) state has been proposed as a vital survival strategy for *Salmonella* under desiccation conditions.

Purpose: This study investigated the survival of *Salmonella* in four types of dried teas under different storage conditions and evaluated the risk of the formation of VBNC *Salmonella* through different brewing methods.

Methods: Culturable and total viable *Salmonella* cells were quantified to assess *Salmonella* contamination in teas after different storage conditions and brewing methods. Culturable *Salmonella* was determined by plating assay. A method that coupled propidium monoazide (PMA) and quantitative PCR was optimized to quantify total viable *Salmonella* cells and determine the VBNC cells formed at each condition.

Results: Under three storage conditions, the number of survived *Salmonella* was highest in teas stored at 4°C and lowest in teas stored at 25°C. After storage of 120 days, culturable *Salmonella* was detected from all samples ranging from 6–7 log CFU/g (4°C storage) to 3–4 log CFU/g (25°C storage). Brewing teas at 75 and 100°C significantly ($P < 0.05$) reduced the number of viable *Salmonella*, but VBNC *Salmonella* formed when brewed at 75°C.

Significance: The findings of this study demonstrated the survival of *Salmonella* in teas at a wide range of temperatures and revealed that storing teas at refrigeration temperature could enhance the survival of *Salmonella* instead of eliminating it. It also illustrated that brewing could not inactivate *Salmonella* in teas at a temperature below 55°C. Thus, teas contaminated with *Salmonella* can still pose a risk to consumers after long-time storage, and thermal treatment delivered during home brewing may not eradicate *Salmonella* in teas.

P1-225 Transfer of *Escherichia coli* and Attenuated *Salmonella enterica T*yphimurium on the Surface of In-Shell Pecans during Harvest

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Introduction: Harvest practices for pecans results in the nuts coming in contact with the ground, potentially for extended periods of time. This presents a contamination risk with foodborne pathogens if present on the ground.

Purpose: The objective of this study was to determine the potential transfer of *E. coli* and *Salmonella* from the ground to in-shell pecans during the harvesting process.

Methods: Plots (1×2 m) were laid out under pecan trees in an orchard at the USDA-ARS Fruit and Tree Nut Research Unit. Each plot was sprayed with 1 liter of either an *E. coli* TVS 353 or an attenuated *Salmonella* Typhimurium inoculum at different concentrations (low: ≈4 log CFU/ml, mid ≈6 log CFU/ml, high ≈8 log CFU/ml). The next day, pecan trees were mechanically harvested. Nut samples from each plot were collected at 1 min, 4 h, and 24 h following harvest. Samples were washed and diluted with 0.1% peptone and plated on to tryptic soy agar with 80 µg/ml rifampicin (LOD=0.3 log CFU/g). Samples were collected in triplicate with three independent replications (n=9).

Results: Inoculation concentration and replication were significant (*P<0.05*) factors that influenced the transfer of *E. coli* and *Salmonella* on pecans. Replicate 3 had significantly less transfer likely attributed to heavy rain or other weather variables. For the high treatments bacterial transfer to pecans ranged from 0.7±0.3 to 4.1±0.2 and 1.3±0.7 to 4.3±0.4 log CFU/g for *E. coli* and *Salmonella* respectively. For the medium treatments transfer, ranged from <0.3 to 1.5±0.1 and <0.3 to 1.9±0.2 log CFU/g for *E. coli* and *Salmonella* respectively. For the low treatments, transfer ranged from <0.3 to 0.4±0.2 and <0.3 to 0.5±0.1 log CFU/g for *E. coli* and *Salmonella,* respectively.

Significance: The bacterial transfer shows the importance of implementing agricultural practices that prevent potential transfer of foodborne pathogens onto the surface of in-shell pecans during harvest.

P1-226 Prevalence, Levels, and Distribution of Shiga Toxin-Producing *Escherichia coli* and *Salmonella* on Raw Almond Kernels from the 2021 California Harvest

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Introduction: The prevalence and levels of pathogens in foods are important inputs for quantitative microbial risk assessment. Previous almond surveys conducted over 9 years, between 2001 and 2013, focused on *Salmonella*.

Purpose: The objectives of this study were to determine the prevalence, levels, and distribution of O157 and non-O157 Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* on raw almond kernels collected from the 2021 harvest in California.

Methods: Single-variety almond lots (n = 601) representing several major varieties were collected from California processors during the 2021 harvest. Each lot was evaluated for the presence of STEC and *E. coli* O157:H7 (375-g subsamples) and *Salmonella* (100-g subsamples). Aerobic plate counts (APC) and levels of coliforms, *Enterobacteriaceae*, yeasts, and molds were determined for a subset of positive and negative lots. Levels of *Salmonella* were determined using a modified FDA BAM 3-tube MPN analysis. A subset of *Salmonella*-negative lots was resampled by enriching multiple (three to 10) 100-g subsamples through sample depletion.

Results: None of the 601 lots was positive for either STEC or *E. coli* O157:H7. *Salmonella* was isolated from 18 lots (3.0%) during initial screening and from eight of these lots during subsequent MPN analysis. In addition, *Salmonella* was recovered from three of 53 initially *Salmonella*-negative lots upon enrichment of an additional 300 to 1,000 g. *Salmonella* levels across all positive lots were estimated to be 0.0016 to 0.011 MPN/g (mean 0.0034 ± 0.0023 MPN/g). Thirty different isolates were recovered representing 14 different *Salmonella* serovars. Where determined, APC and levels of *Enterobacteriaceae*, yeasts, molds, and coliforms were statistically similar ($P > 0.05$) between the initially *Salmonella*-positive and *Salmonella*-negative lots.

Significance: STEC was not detected in the raw almonds surveyed; the prevalence and levels of *Salmonella* in raw almonds was low, which is consistent with previous surveys conducted.

P1-227 Differential Attachment between Wildtype *Salmonella enterica* Serotype Enteritidis and Its Mutant Cells to Almond Seeds

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Introduction: *Salmonella enterica* Enteritidis has been a causative agent of outbreaks associated with low-moisture foods. Pathogen attachment is critical for initial establishment on low-moisture foods as well as subsequent survival under desiccation. In an early study of our laboratory, five knock-off mutants of *S*. Enteritidis PT30, a raw almond outbreak strain, were obtained using mini-Tn*10* transposon mutagenesis.

Purpose: This study assessed the attachment ability of the five knock-off mutants of *S*. Enteritidis PT30 along with their wildtype parent strain to almond seeds.

Methods: Sterilized almond seeds (2 g) were inoculated with sandy soil (20 g) containing 10^6 or 10^7 CFU/g of lyophilized cells of wildtype and mutant *Salmonella* cells at room temperature for 1 h with vigorous mixing. Almond seeds with attached *Salmonella* cells were then soaked in 5 ml phosphate buff-

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ered saline (PBS) at 4˚C for 0 or 24 h. The number of *Salmonella* cells in PBS was enumerated on tryptic soy agar before and after the soaking process. The percentage of cells in contaminated sandy soil attached to the almond seeds was subsequently calculated.

Results: Type III error analysis showed *Salmonella* culture is a significant factor but seed soaking time is not a significant factor affecting *Salmonella* attachment to almond seeds. *Salmonella* mutants had a significantly lower levels of attachment than those of the wildtype parent at both inoculation levels, indicating that the defective genes encoding for aldehyde dehydrogenase, transporter, porin OmpL, ribbon-helix-helix protein from CopG family, cysteine desurfurase in the five knock-off mutants play critical role in attachment to raw almond seeds. Extended seed soaking from 0 to 24 h did not significantly increase the release of *Salmonella* cells from almond seeds to PBS.

Significance: Therefore, products of the mutated genes could serve as potential targets for the development of antimicrobial interventions to prevent *Salmonella* contamination on almond seeds, and perhaps other seeds.

P1-228 Growth and Biofilm Formation Ability of *Salmonella* Strains Isolated from Pistachios

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❖ ***Developing Scientist Entrant***

Introduction: The recovery of limited number of *Salmonella* strains from California pistachios over the past 15 years is indicative of stable populations that have persisted in production, harvest, or postharvest environments.-

Purpose: To compare the growth and biofilm formation of *Salmonella* strains isolated from California pistachios.

Methods: Nine *Salmonella* strains representing six serovars repeatedly isolated from California pistachios were evaluated: Agona (1), Enteritidis (2), Montevideo (2), Senftenberg (1), Liverpool (2), and Worthington (1). Individual *Salmonella* strains were inoculated (2.7 log CFU/mL) into a sterile pistachio hull slurry and incubated at 30°C for 48 h; populations were determined by plating onto tryptic soy agar and CHROMagar™ at regular intervals from 0 to 48 h. Average counts were statistically analyzed using ANOVA followed by Tukey's test. A crystal violet (CV) microtiter assay was used to assess biofilm formation after 96 h at 25°C by measuring optical density (OD_{600}) of resuspended CV (0.5%). Three biological replicates were performed for each phenotypical test.

Results: Pistachio hull slurry supported rapid growth of eight *Salmonella* strains at 30°C; populations increased significantly ($P < 0.05$) by 5.58 to 5.90 log after 24 h. The growth of one *Salmonella* Enteritidis strain (SE1) was significantly lower in the first 28 h compared with the others. At 40 h, populations of all strains were not significantly different; populations had increased to 8.48 to 8.75 log CFU/mL after 48 h. Seven of the nine strains produced moderate biofilms; one *Salmonella* Liverpool strain produced strong biofilms, and SE1 formed weak biofilms.

Significance: Most *Salmonella* strains multiply rapidly in pistachio hull extract and are moderate to strong biofilm formers. Both phenotypic characteristics may contribute to the persistence of *Salmonella* strains in the pistachio processing environment.

P1-229 Copper-Resistance Genotypes and Phenotypes of *Salmonella enterica* Isolated from California Pistachios

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❖ ***Developing Scientist Entrant***

Introduction: The recovery of a narrow range of *Salmonella enterica* serovars from California pistachios over 15 years indicates the presence of persistent populations in the production and processing environments. The application of copper as a foliar fertilizer has been suggested as one mechanism that may limit *Salmonella* diversity in pistachios.

Purpose: To compare copper-resistance genotypes and phenotypes of representative *Salmonella* isolated from pistachios.

Methods: Nine *Salmonella* strains (six serovars) isolated from pistachios were selected: Agona (1), Enteritidis (2), Montevideo (2), Senftenberg (1), Liverpool (2), and Worthington (1). All strains, excluding *Salmonella* Enteritidis, were considered persistent strains (isolated for ≥3 years from more than one avenue [e.g., surveys, outbreak investigations, or routine sampling]). The presence of the copper homeostasis and silver resistance island (CHASRI) was determined by comparing the genetic sequences of the assembled genomes against an annotated reference sequence of *S. enterica*. The copper MICs were determined by measuring the optical density (OD) of *Salmonella* suspensions mixed with different concentrations of CuSO4 or Cu-EDTA and incubating under anaerobic or aerobic conditions at 37°C for 24 h. MICs, interpreted according to Clinical and Laboratory Standards Institute guidelines, were conducted in duplicate and repeated in three independent trials.

Results: CHASRI sequence was identified in three of the seven persistent strains (one each *Salmonella* Montevideo, Senftenberg, and Worthington). The MIC for Cu-EDTA was ≥120 mM for all strains under both aerobic and anaerobic conditions. The MIC for CuSO4 was 15 mM for all strains under aerobic conditions, but 15 mM and 7.5 mM for strains with and without CHASRI, respectively, under anaerobic conditions.

Significance: The presence of CHASRI conferred resistance to copper under anaerobic conditions. CHASRI was not present in all persistent strains, suggesting that additional genotypic and phenotypic factors influence the persistence of *Salmonella* in pistachio environments.

P1-230 Evaluation of the Influence of Pre-Conditioning of Contact Surfaces on Dry Adhesion of *Salmonella*

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Introduction: Low moisture foods (LMF) are defined as a food category with water activity (a_w) ≤0.85. Although *Salmonella* cannot grow at a_w <0.94, salmonellosis outbreaks associated to LMF have been reported in last decade.

Purpose: The aimed of this study was to evaluate the influence of different conditioning matrices on the dry adhesion of a pool of four *Salmonella* strains (Muenster, Javiana, Oranienburg and Miami) on stainless steel (SS) and polypropylene (PP) coupons.

Methods: Both surfaces were pre-conditioned with sterile soil, powdered milk and sterile crushed peanut skin (CPS) by agitation at 200rpm for 24h. Then, the coupons were transferred to sterile vials containing soil inoculated with *ca.* 6 log cfu/g of the *Salmonella* pool and kept in contact with it for up to 7 days at 25 ºC.

Results: In general, PP showed greater adhesion of *Salmonella*, however, most of the time there was no significant difference between the surfaces (p>0.05). The highest initial *Salmonella* adhesion was observed on both surfaces pre-conditioned with CPS, 3.1 and 2.3 log cfu/cm², respectively. In contrast, the lowest adhesion was verified on PP pre-conditioned with powdered milk and on SS pre-conditioned with soil. After 7 days, the greatest *Salmonella* counts were obtained on PP pre-conditioned with soil and on SS pre-conditioned with CPS, whereas the smallest *Salmonella* populations were recovered from the surfaces pre-conditioned with powdered milk, 1.0 log cfu/cm² for PP and 0.5 log cfu/cm² for SS. Indeed, the contact time of the coupons with the soil influenced (p<0.05) the *Salmonella* adhesion on both coupons pre-conditioned with powdered milk, and on PP pre-conditioned with CPS.

Significance: Therefore, the results suggest that the presence of certain dry residues can favor the dry adhesion of *Salmonella* on materials commonly used in manufacturing plants.

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P1-231 Impact of the Water Activity and Transfer Vehicle on the Dry Adhesion of *Salmonella*

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Introduction: The *Salmonella* cross-contamination is a significant issue faced by the food industry. Even in manufacturing plants with low water activity (a_w), cross-contamination can occur, resulting in cell adhesion on equipment surface.

Purpose: This study aimed to evaluate the influence of dry transfer vehicles and their a_w on the *Salmonella* adhesion on stainless steel (SS) and polypropylene (PP) coupons.

Methods: Two dry transfer vehicles (crushed peanut shell and soil) with three a_w values (0.55; 0.65 and 0.85) were tested. The vehicles were inoculation with *ca.* 6 log cfu/g of a pool of four *Salmonella* strains (Muenster, Javiana, Oranienburg and Miami). Then, to simulate a dry contamination the SS and PP coupons were transferred to a vial with the inoculated vehicles and kept in desiccators with controlled a_w for up to 7 days at 25ºC. After that, the *Salmonella* count on the coupons was determined.

Results: For all tested conditions on SS, *Salmonella* with counts close to the limit of detection (0.3 log cfu/cm²) was observed. On PP the a_w of the transfer vehicle influenced the *Salmonella* adhesion (p<0.05). On PP coupons contaminated by crushed peanut shell, the count was 1.8, <0.3 and 0.6 log cfu/cm² for a_w 0.85, 0.65 and 0.55, respectively. In contrast, on PP coupons contaminated by soil the *Salmonella* counts ranged from 1.1 to 1.9 log cfu/cm² at day 0, and there was no significant difference (p>0.05) among the a_w values. However, the protocol using soil with a_w 0.55 was the only treatment where the *Salmonella* counts remained above the limit of detection after 7 days at 25 ºC (p<0.05).

Significance: The data show that dry residues can cause cross-contamination and result in *Salmonella* cell adhesion especially on PP surfaces. It reinforces the needed to implement prerequisite programs with strict control in the LMF industry.

P1-232 Spoilage in Plant-Based Meat Alternatives, and How to Achieve Shelf-Life Extension

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Introduction: The market for plant-based meat alternatives is growing and innovating rapidly to satisfy consumer demands for healthier and more sustainable foods. Expectations are ever-increasing regarding taste, quality, convenience, food-safety and shelf-life. Consumer concerns regarding food-safety and shelf-life of plant-based products has been exacerbated by consumers unfamiliarity with these products along with the dearth of data available.

Purpose: Establish the food-safety and spoilage microbiological characteristics of plant-based meat alternative products on the market.

Methods: 40 plant-based meat alternative products were analyzed. Samples were sourced from the market and stored at 5˚C. Product characteristics were determined on date of purchase. Triplicate samples were taken on the expiry date and 7 days post-expiry. 16S qPCR was used to quantify bacterial load. 16S rDNA amplicon sequencing was performed for population analysis. Relative abundance plots were created in R, using OTUs (operational taxonomic units) classified to the genus level. Median values of abundance were calculated for each meat sample per timepoint across the replicates sequenced. Any OTU's present at <1% were collapsed into a category termed "Other".

Results: Overall, there was a wide diversity of product characteristics – pH, a_w, salt, moisture. Shelf-life of the products was short, approximately 10-12 days. Lactic Acid Bacteria (LAB) were the dominant microflora with *Leuconostoc* and *Lactobacillus* the dominant genera. Enterobacteriaceae, *Pseudomonas*, yeast & mould were present at comparatively low levels. Particular products exhibited different profiles with *Brochothrix* and *Pseudomonas* being the dominant genera. Some products comprised a very homogeneous population structure, while others were quite diverse. Notably, the manufacturing site was a significant factor in microbiome development.

Significance: This work demonstrates the diversity of microbial populations found in plant-based meat alternative products and their dynamics. This knowledge will be critical to optimise preservation strategies to ensure food-safety and enhance the shelf-life of plant-based meat alternative products.

P1-233 Characterization of Bacterial Diversity on Spinach from Different U.S. Locations over Shelf Life

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❖ ***Developing Scientist Entrant***

Introduction: An improved understanding of bacteria contributing to spoilage of produce may help to better predict produce shelf life and reduce food waste.

Purpose: The goal of this study is to comprehensively characterize culturable bacteria found on baby spinach at harvest and over shelf life, in order to assess changes in the abundance and diversity of the microbial community. A collection of isolates obtained from produce also allows for research that requires greater depth than 16S rRNA metagenomics, including cold growth and enzyme production.

Methods: Approximately 2,400 bacterial isolates were collected from spinach (n=20 sample collections of spinach) over December 2021 to December 2022. Samples were collected from a single supply chain that receives spinach from two locations (Arizona and California). For each sample collection, isolates were collected at three time points: harvest, 7 days post packaging, and 22 days post packaging. All isolates were characterized by 16S rRNA gene sequencing. Allelic types (AT) were assigned based on the 16S rRNA sequence data.

Results: The most common genera recovered were *Pseudomonas, Pantoea* and *Erwinia* (48, 18, and 11% of isolates, respectively). These three genera were also the most abundant for each location and two of the three time points; for the "harvest" time point *Bacillus* replaced *Erwinia* as the third most common genus. Relative abundance of *Pseudomonas* was significantly higher in Arizona samples (P<0.005) via Mann Whitney U test. A PERMANOVA using the Bray-Curtis dissimilarity matrix using AT data indicated timepoint and location were significant (P<0.001), but not the interaction of day and location.

Significance: *Pseudomonas, Pantoea, and Erwinia* were identified as the predominant culturable organisms throughout shelf life across spinach grown in two locations in the US, providing initial evidence that strategies targeting reduction or growth inhibition of these genera may increase shelf life.

P1-234 Controlling Yeast and Mold Spoilage to Increase Shelf Life in Beverage Applications Using Citrus Extracts

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Introduction: Refreshing beverage applications include flavored waters, juice beverages, soft drinks, and energy drinks, that, due to their nutrients, high water activity (a_w) and low pH, refreshing beverages are prone to microbial spoilage, especially with yeast and mold.

Purpose: To investigate the impact of pH, Aw and different antimicrobials on the rate of spoilage of common beverage yeast and mold strains in a model juice base targeting specific strains based on their growth and resistance kinetics.

Methods: 100% clarified orange juice was prepared to varying pHs (2-4 with HCl and NaOH), a_w (0.85-0.99 with sucrose), potassium sorbate levels (0-0.1%), and Kerry citrus extract levels (0-0.1%). Yeast strains tested included*: Zygosaccharomyces bailii, Saccharomyces cerevisiae, Dekkera bruxellensis,* and *Brettanomyces naardensis*. Mold strains tested included: *Aspergillus niger, Penicillium commune, Cladosporium cladosporioides,* and *Paecilomyces fulvus*.

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Samples were inoculated with individual strains to a concentration of 5-log cfu/g yeast or 5-log spores/g mold and incubated at 25°C for up to 8 weeks. Minimum inhibitory concentrations (MIC) were determined and lag time was modelled using a Gompertz function.

Results: Z. bailii. and *P. commune* were the most resistant yeast and mold genus, respectively, while other yeast and mold species showed sensitivity to the ingredients tested at lower levels. Efficacy against both resistant genera was observed with the citrus extract, with the MIC of *Z. bailii* being 0.015% and *P. commune* being 0.03%. pH had minimal effect on lag time of yeasts and molds tested. Decreasing a_w had the most significant effect on *Paecilomyces fulvus*, with no growth observed below a_w 0.93.

Significance: Understanding the impact of antimicrobials and product proximates on the rate of spoilage in beverages will help product developers formulate beverages with increased food safety and quality

P1-235 Assessment of Natural Extracts for Inhibition of Preservative Resistant Yeast – *Zygossacharomyces bailii* Outgrowth

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Introduction: Zygosaccharomyces bailii is well documented in literature to be resistant against weak acid-based preservatives, such as potassium sorbate and sodium benzoate. As Z. bailii is a common spoilage microorganism in foods and beverages, particularly in high acid products such as salad dressings, mayonnaise, ketchup, carbonated soft drinks (CSDs), and sweet syrups, alternative solutions are required to control Z. bailii spoilage.

Purpose: To evaluate the performance of a citrus extract, smoke fraction on Z. bailii.

Methods: Ten varying concentrations of potassium sorbate (0-0.1%), sodium benzoate (0-0.1%), citrus extract (0-0.15%) and smoke fraction (0-4%) were prepared in potato dextrose broth (PDB). Samples were pH adjusted to either pH 4 or 6 using hydrochloric acid. Samples were aliquoted into 100-well honeycomb plates and growth curves generated (Bioscreen-C Pro, 25°C). Variables were tested in duplicate. The Modified Gompertz equation was used to determine maximum growth rate, and standard deviations calculated for duplicate measures. Wells with less than 0.2 OD 600nm change were considered to have no growth, and used as basis for minimum inhibitory concentration. Sensory evaluation of microbially active levels of the natural extracts was conducted on a CSD (pH 3.5), and a beverage sauce (pH 6.0).

Results: At pH 4 and 6, Z. bailii inhibition was achieved with either 0.01% citrus extract or 0.35% smoke fraction ($p<0.05$). At pH 4, inhibition was observed with 0.04% potassium sorbate, but at pH 6, all levels tested supported growth. All levels of sodium benzoate tested supported Z. bailii growth at pH 4 and 6. The use of the natural extracts tested at microbially active levels were sensory acceptable in the CSD and beverage sauce.

Significance: This work substantiates the use of several natural extracts for *Z. bailii* inhibition across food and beverage applications with varying pHs, and thus provide an alternative to the use of sorbates and benzoates.

P1-236 Control of *Listeria monocytogenes* in Plant-Based Cheese Sauce Using Cultured Sugar and Vinegar System

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Introduction: In the recent years, consumer interest in the consumption of dairy-free products has increased, as well as the consumer demand around natural and clean label foods. The food safety control for *Listeria monocytogenes* requires preservation system in the formulation. This study demonstrates the use of a clean label cultured sugar and vinegar (CSV) system to extend shelf life of plant-based cheese sauce without compromising product quality and safety.

Purpose: Investigate the growth inhibitory effect of a cultured sugar and vinegar against *Listeria monocytogenes* in plant-based cheese sauce at refrigeration temperature (4ºC) over 63 days.

Methods: The effect of 1.1% CSV, 0.85% commercial market sample, and 0.1% sorbic acid were investigated. Samples without antimicrobial were included as negative control. Each treatment was formulated to the final pH of 5.7. All treatments were inoculated to the target level of 10^4 CFU/g of target microorganism (LM101M, LM108M, LM310, LM301) and stored at 4ºC. Duplicate samples of each inoculated variable were plated once a week on selective agar (Modified Oxford Agar) to monitor *L. monocytogenes* counts over the shelf life.

Results: At day 63, the number of *L. monocytogenes* population was 2.39 log CFU/g (STD=0.12) in samples containing 1.1% vinegar cultured sugar and 6.32 log CFU/g (STD=0.04) in negative control samples. The population of *L. monocytogenes* decreased by 1.51 log CFU/g in samples containing commercial market sample and by 1.39 log CFU/g in samples containing sorbic acid over the course of the study (63 days).

Significance: The result of this study demonstrates the efficacy of Cultured sugar and vinegar to control and even decrease the *L. monocytogenes* population in plant-based cheese sauce, showing an even higher decrease in counts compared to sorbic acid over the course of shelf life.

P1-237 The Use of Fermentation Based Fermentate to Control Spoilage Microorganisms in Cottage Cheese

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Introduction: Various technologies applied in the dairy industry to control the spoilage of cottage cheese create challenges with regards to consumer acceptability which requires the industry to provide natural solutions to meet consumer's needs for clean label products in cottage cheese.

Purpose: Investigate the growth inhibitory effect of cultured skim milk against yeast and mold in low-fat and non-fat cottage cheese at refrigeration temperature (4ºC) over 60 days.

Methods: Cottage cheese were produced by incorporating 0.3% cultured skim milk fermentate (Durafresh Product Line) and 0.25% commercial market sample. Samples with no antimicrobial were included as negative control. All treatments were inoculated to the target level of 10^3 CFU/g of yeast and mold cocktail individually and stored at 4ºC. Duplicate samples of each inoculated variable were sampled onto selective media (Potato Dextrose Agar) once a week for enumeration of yeast and mold over the shelf life.

Results: In low-fat cottage cheese all treatments inoculated with yeast exceeded spoilage limit (6 Log CFU/g) within 35 days, with 0.3% cultured skim milk-containing samples exhibiting the lowest yeast growth (1.08 Log CFU/g) over 28 days compared to other treatments. Samples of all treatments showed visible mold growth by day 21, with samples formulated with 0.3 % cultured skim milk showing the lowest mold growth (0.1 Log CFU/g) over 14 days. In fat-free formula, samples formulated with 0.3% cultured skim milk exhibited 2.49 Log CFU/g increase in the number of yeast over 63 days whereas samples containing commercial market sample exceeded 6 Log CFU/g within 35 days of storage at 4ºC.

Significance: Cultured skim milk fermentate treatment was efficacious against the outgrowth of spoilage organisms in cottage cheese. The Cultured skim milk fermentate treatment is substantially more effective in controlling yeast in fat-free formula compared to commercial market sample over a period of 63 days.

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P1-238 Effect of pH, Solids Content and Storage Temperature on Post-Pasteurization Spoilage of Tomato Paste

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Introduction: Spoilage of thermally processed tomato paste due to spore-forming spoilage organisms are of great economic concern to the industry, especially if the natural acidity is to be maintained in the finished product. Common perception in the industry is that this product does not support the growth of these spoilage organisms when their pH and solids content are controlled but no scientific literature is available to support this.

Purpose: The objective of this study is to evaluate the effect of different pH levels, solids content and storage temperature on growth of selected spore-forming spoilage organisms that may affect the shelf-stability of tomato paste.

Methods: Tomato paste with varying pH levels (4.4, 4.6 and 4.8), solids content (5, 12 and 18 per cent) were heat treated, cooled and inoculated with 4-5 log CFU/g cocktails of selected butyric acid anaerobes and aerobic mesophilic organisms. Replicate samples incubated at 30°C and 40°C were evaluated periodically for growth (>1 log increase) and other quality parameters over a period of 8 weeks. Target organisms were enumerated by plating and genetically identified at the end of study period.

Results: Lowering the pH was found to be the controlling factor against growth of spoilage organisms irrespective of solids content. Higher levels of solids content alone was not able to suppress the growth though the levels of growth were found to be low at higher solids level. Higher incubation temperature was found to favor pH increase and increased growth.

Significance: The study demonstrated that good control over GMPs and acid levels of finished product are critical in controlling incidents of spoilage in tomato paste. Higher solids content alone cannot deter the growth of spoilage organisms. Timely harvest, clean handling and optimum storage conditions are critical in reducing thermophilic spoilage of thermally processed acid products like tomato paste.

P1-239 Spoilage and Food Waste: Assessing the Role of Predictive Modeling and Food Date Labeling

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Introduction: A significant portion of food produced for human consumption is wasted annually. Food waste can be attributed to a number of actions performed during food processing, post-processing, transportation, storage, and consumer handling. However, spoilage remains a major contributor to food being lost or wasted.

Purpose: The aim of this study is to review the role of food spoilage in food waste generation and discuss the role of advanced scientific techniques and smarter date labeling in mitigating food waste.

Methods: We conducted an extensive search of scientific literature on Google Scholar, Web of Science, and the National Center for Biotechnology Information's PubMed databases using various combinations of the search terms "food waste," "microbial contamination," "cross contamination," "food spoilage," "date labeling," "novel packaging," "novel technologies to reduce spoilage," and "predictive modeling." We performed a comprehensive review of the selected studies to identify novel strategies to mitigate food spoilage and food waste.

Results: Although ~31% of food produced in the U.S. is lost or wasted annually, the exact proportion attributed to microbial food spoilage remains unknown. Herein, we reviewed 40 studies on novel technologies and strategies to combat spoilage-related food waste. Quantitative microbial spoilage risk assessment is increasingly being proposed as a needed step to predict and prevent the occurrence of spoilage to minimize food loss and waste. Increasing transparency and consumer awareness regarding different food date labels, and combining the same with novel sensing and packaging technologies, could also contribute to reduced food waste at the pre-retail, retail, and consumer scales.

Significance: With the increase in demand for food comes a corresponding need to increase the safety and security of our food supply. This review highlights the role played by food spoilage in the generation of food waste, and identifies novel methods to mitigate spoilage-related food waste and improve sustainability in the food chain.

P1-240 Predictive Model for Growth of Gas-Producing *Leuconostoc* spp. in Deli Meat

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Introduction: Spoilage of MA-packed deli meat resulting in recalls from the market is often caused by unwanted gas production. Development of models predicting the growth of gas-producing microorganisms will make it possible to minimize spoilage and reduce food waste of deli meat.

Purpose: Isolation and identification of the causative microorganisms for blown deli meat and developing a mathematic model to predict their growth.

Methods: Blown packages of deli meat were obtained from retailers. Gas-producing organisms were isolated and identified with whole genome sequencing (WGS), and 11 strains were chosen for developing the predictive growth model. Data for the model was generated by inoculating sliced deli meat with varying combinations of pH, NaCl, nitrite, Na-lactate, and Na-acetate. The deli meat was manufactured at in-house pilot plant facilities, sliced under sterile conditions, inoculated with the 11-strain *Leuconostoc* cocktail, MA-packaged (30% CO_2/70% N_2), and stored at temperatures between 3°C and 8°C.

Results: Spoilage resulting in blown packages was mainly caused by *Leuconostoc carnosum* and *Leuconostoc mesenteroides.* The challenge test showed that high concentrations of preservatives are needed to inhibit growth of the *Leuconostoc* cocktail. For example, at pH 6, an 8-log growth within 60 days at 3°C was observed, although the deli meat was preserved with 4% NaCl, 3% Na-lactate, and 0.5% Na-acetate. Furthermore, the studies showed that gas production did not occur until the *Leuconostoc* count had been at maximum level for 1-2 weeks. Validation of the model (n=59) showed that it was acceptable with a bias factor (B_f) of 0.90 and an accuracy factor (A_f) of 1.38. The model will be made publicly available at DMRIPredict in 2023 (www.dmripredict.dk).

Significance: A model predicting the growth of gas-producing spoilage organisms will enable deli meat manufacturers to optimize their preservation strategy, thus prolonging product shelf life and minimizing spoilage, recalls, and food waste.

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P1-241 Prevalence of Microorganisms Related to Volatile Basic Nitrogen Production in Beef

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Introduction: Volatile basic nitrogen (VBN), such as ammonia and trimethylamine, is one of the indicators used to determine meat spoilage. Levels of VBN increase with the degradation of proteins by microorganisms as the freshness of meat decreases.

Purpose: This study investigated the prevalence of microorganisms related to VBN production in beef.

Methods: Total-VBN and total aerobic bacteria (TAB) were measured in 70 beef samples (34 samples of sirloin and 36 samples of top rounds). To investigate the relationship between beef microorganisms and VBN, microbiota were analyzed for beef samples with high VBN and low VBN groups. In addition, 7 isolates (TAB, Coliform, lactic acid bacteria, *Pseudomonas* spp., Enterobacteriaceae, fungi, and psychrophilic bacteria) were inoculated in beef at 4-5 log CFU/g. The number of microorganisms, VBN, and pH were then measured during the storage at 10°C for 7 days. This experiment was repeated three times, and Spearman's correlation coefficient was analyzed between microbial count and VBN change.

Results: For the microbial composition analysis, Firmicutes and Proteobacteria were in common at the phyla level, however, only low-VBN group showed Actinobacter. Also, beef samples with high-VBN group showed high abundance of lactic acid bacteria, *Dellaglioa*, *Leuconostoc*, *Latilactobacillus*, and *Carnobacterium* at the genus level. Regarding α-diversity, samples with low-VBN group contained more diverse microbiota. In beef inoculated with the iso-

lates, *Pseudomonas* spp. group showed the highest increase in pH and VBN. As a result of the correlation analysis between the microorganisms and VBN, lactic acid bacteria, *Pseudomonas* spp., and fungi showed higher correlations than the other groups ($P<0.05$).

Significance: This result indicates that lactic acid bacteria, *Pseudomonas* spp., and fungi play a role in producing VBN, and thus, these microorganisms need to be controlled in beef.

P1-242 Efficacy of Commercially Available Low Sodium Organic Acid Salts Against *Lactobacillus sakei* in a Low Sodium Hot Dog Formulation

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Introduction: *Lactobacillus sakei* is a major meat spoilage micro-organism at refrigeration temperature, resulting in generation of off-odors and slime. Consumer interest in low sodium options illicit the need for potassium-based antimicrobials. Highly effective potassium based acetic acid salts would be a viable alternative for meat applications.

Purpose: To evaluate the efficacy of sodium and potassium based organic acid salts against *L. sakei* in frankfurters at 2, 4, 7°C for up to 120 days incubation.

Methods: Frankfurters (1300 per treatment) of eight organic acid treatments (0.25-0.75% Provian K, 0.06-0.16% sodium acetate and 1.17-3.25% potassium lactate) and control (no antimicrobials) were inoculated (2-3 $\log_{10}$ CFU/g) with *L. sakei*, massaged in bags to disperse inoculum, vacuum packaged and stored at 2, 4, 7°C for up to 120 days. At each sampling, sample homogenate was plated onto deMan, Rogosa, Sharpe agar for enumeration. Spoilage threshold was considered as 6 log CFU/g, and treatment performance was compared using one-way ANOVA ($P < 0.05$). Primary modeling was used to model growth over time and estimate day of spoilage.

Results: Inoculation level of 2.5 log CFU/g of *L. sakei* was achieved on day 0 for all treatments. Overall, 3.25% potassium lactate and 0.75% Provian K were very similar in their antimicrobial performance. Control treatments showed fastest outgrowth of *L. sakei* at all temperatures, reaching 6 $\log_{10}$ CFU/g on 34, 24 and 10 days storage at 2, 4 and 7 °C, respectively. Provian K (0.75%) showed significantly enhanced shelf life compared to control ($P \leq 0.0032$) at these storage temperatures, imparting 14, 11 and 2 days shelf life extension at 2, 4 and 7 °C, respectively.

Significance: Provian K exhibited superior antimicrobial efficacy against *L. sakei* in processed meat at a 3 to 5 times lower use level compared to potassium lactate-based preservatives, providing meat processors with cost-efficient solutions.

P1-243 Genotypic Characterization of *Leuconostoc* Spoilage Strains Isolated from Ice Cream Mix and Milk Syrup

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❖ ***Developing Scientist Entrant***

Introduction: Sweetened dairy products such as ice cream mix and milk syrup have been implicated in spoilage events associated to lactic acid bacteria, mainly *Leuconostoc*. Characterization of strains will allow the identification of genotypes associated to food spoilage.

Purpose: Determine the genetic diversity of *Leuconostoc* strains causing spoilage in ice cream mix and milk syrup.

Methods: *Leuconostoc* strains were isolated from spoiled and freshly processed samples of ice cream mix (n= 41) and milk syrup (n= 52) of a processing plant. *Leuconostoc* strains were confirmated at the genus level by a PCR test. The *Leuconostoc* strains were characterized by RAPD's assay using primers directed to dextran sucrase gen.

Results: Fourty eigth strains were obtained from spoiled (17) and freshly processed (31) samples of ice cream mix and milk syrup. The 95.8 % (46) were identified as *Leuconostoc*. From 46 *Leuconostoc* strains genetically characterized, 18 genotypes was found. Genotype X was the most frequent genotype (21.7 % of the strains), which were isolated from deteriorated (50 %) and freshly processed (50 %) samples. On the other hand, genotypes II and III were isolated only from spoiled samples, while the other genotypes were isolated only from freshly processed samples. This information provides a broad overview of the *Leuconostoc* diversity in production process of products such as ice cream base and milk syrup. Genotypes II, III and X could be the responsable of these foods spoilage.

Significance: Genetic diversity of spoilage strains provides information to understand the spoilage process.

P1-244 Impact of Carbon Dioxide Partial Pressure on the Radial Growth of Fungi in a Dairy Environment

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Introduction: Uncontrolled growth of filamentous fungi can lead to waste of dairy products, among others. Increasing consumer demand for preservative-free products accelerated the development of alternative preservation strategies such as Modified atmosphere packaging (MAP). Nonetheless, current predictive microbiology tools lack of data and mathematical models to predict mold growth under MAP conditions.

Purpose: The present study evaluated the impact of dissolved carbonic acid on the radial growth response of molds isolated from dairy products or environment.

Methods: A specific device was developed to monitor fungal growth under static modified atmosphere. Radial growth rates were estimated as a function of CO_2 partial pressure and, more specifically, dissolved carbonic acid for fourteen adjuncts and/or fungal spoilers isolated from dairy products or the dairy environment. Modelling provided biologically significant parameters, such as carbonic acid concentration at which the growth rate is reduced by 50% ($[\textit{Carbonic acid}]_{50}$), for spoilage prediction purpose.

Results: A significant diversity was observed in the growth rates of the selected strains depending on carbonic acid. Independently of pH, most strains were sensitive to the total carbonic acid (H2C03 + HCO3-) and only *Geotrichum candidum* and *M. circinelloides* strains were sensitive to undissociated carbonic acid. Among the fourteen strains, *P. bialowiezense* was the most sensitive to carbonic acid; no growth was observed at 0.25 $mmol.L^{-1}$ of total carbonic acid. *Mucor lanceolatus* was the least sensitive strain to carbonic acid with a $[\textit{Carbonic acid}]_{50}$ of 0.70 $mmol.L^{-1}$ of total carbonic acid. Interestingly, low carbonic acid concentrations improved the growth of *Penicillium expansum*, *Penicillium roqueforti* and *Paecilomyces niveus*.

Significance: New mathematical models are proposed to describe the impact of carbonic acid on fungal growth rate based on the carbonic acid in the culture medium rather than the carbon dioxide in the headspace because carbonic acid can be involved in the modification fungal cell physiological processes.

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P1-245 Comparative Genomic Analysis of Strains with and without Potential to Cause Ropy Defect in Milk Reveals No Association between Genetic Content and Ropy Phenotype

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Introduction: Ropy milk is a body defect that results from production of microbial exopolysaccharides (eps), most commonly produced by Gram-negative bacteria that are introduced into milk as post-pasteurization contaminants (PPC). Although this spoilage phenomenon is a long-standing problem of economic importance for fluid milk processors, limited information on bacterial species and strains, their phenotypic properties and ultimately specific genes that may be attributed to spoilage incidents are available.

Purpose: This study aimed to characterize and compare five bacterial strains including *Rahnella inusitata* (n=2) and *Klebsiella pneumoniae subsp. pneumoniae* (n=3) previously isolated from milk samples with and without ropy defect.

Methods: The five bacterial strains were characterized for growth and increase of viscosity in UHT milk at two different temperatures (6 and 21°C); performed in three biological and two technical replicates, and compared using ANOVA and post hoc Tukey's test. Additionally, all five strains were genome sequenced and genomic comparison was performed.

Results: Growth analysis in UHT milk at 21°C revealed increase of bacterial counts of all isolates by at least 4-log over 48 h while only two strains of *K. pneumoniae subsp. pneumoniae* and one strain of *R. inusitata* were able to cause the ropy defect. At 6°C, the growth was observed for only the two *R. inusitata* strains, of which one strain caused the ropy defect. Comparative genomics identified eps gene clusters in genomes of all five strains; however, the number and the composition of eps biosynthesis gene clusters varied among all studied strains. Furthermore, no clear-cut relationship between the ropy phenotype in milk and gene contents were found when strains of the same species were compared.

Significance: Our results indicate that the potential of *R. inusitata* and *K. pneumoniae subsp. pneumoniae* to cause a ropy defect in milk is strain-dependent and that this potential cannot be explained by simple differences in genetic content.

P1-246 Soleris® Rapid Method for Determination of Psychrotrophic Microorganisms in Raw Milk

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Introduction: The content of psychrotrophic microorganisms is one of the vial parameters for identifying the quality of raw milk. These bacteria can produce thermal-stable protease and lipase during metabolism, and these enzymes may lead to several quality issues in diary products, including shorten the shelf life, flavor change, milk clumps and protein aggregation.

Purpose: To establish an early warning method for high content of psychrotrophic microorganisms in raw milk with the Soleris® technology.

Methods: We collected 445 batches of raw milk from different sources and factories around Hangzhou, Zhejiang. The cell pellet was collected by centrifuging each raw milk sample and eluted into Soleris test vials to perform the test. The standard curve between detection time and the log value of psychrotrophic microorganisms were established. The feasibility and accuracy of this method were investigated with all batches of milk, and psychrotrophic microorganisms higher than 3×10^4 cfu/mL were set as risk samples.

Results: Considering the source location, transport time and previous data, these raw milk samples were classified into 3 groups. According to the standard curve, all three coefficients of determination (R) are larger than 0.8. The accuracy range is 86%-93%, and the feasibility range is 62.5%-88.89%, which presents a high consistency with reference method (SN/T 2552.4-2010). This rapid method could warn the risk of high content of psychrotrophic microorganisms within 8 hours. In addition, a two-class sampling method was developed, and the optimized parameter was n=9, c=1. When the psychrotrophic microorganisms is larger than 2.7×10^4 cfu/mL, there are 95% confidence to reject this sample, and when the psychrotrophic microorganisms is larger than 1.0×10^3 cfu/mL, there are 95% confidence to accept this sample.

Significance: The Soleris rapid method offers an alternative 24-hour psychrotrophic Microorganisms solution of raw milk with high accuracy and feasibility comparing to reference method.

P1-247 The Effect of Organic Acid-Based Antimicrobials on Controlling *Listeria monocytogenes* Outgrowth in Smoked Salmon at Retail Simulated Refrigerated Storage Conditions

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Introduction: *Listeria monocytogenes* is a threat for RTE food products like smoked salmon, with multiple recalls after outbreaks of listeriosis in recent years.

Purpose: This research shows *Listeria monocytogenes* inhibition in commercial cold smoked salmon products with addition of acetates and lactate during temperature-abused storage of 28 days.

Methods: Six different treatments of smoked salmon were prepared: control (no preservative), 0.61% sodium acetate, 0.61% Provian K, 0.61% Provian N and 1.63% sodium lactate 60%. The slices were inoculated with a cocktail of three *L. monocytogenes* strains at 2 to 3 logCFU/g. The inoculated smoked salmon slices were vacuum-packed and stored at a consecutive temperature profile of seven days at 2°C, then 12 days at 7°C and finally nine days at 9°C, representing the storage period during production, retail and at the consumer level. Samples were plated in triplicate on PALCAM agar (*Listeria* counts) at regular time points during storage. At t=0 and after 28 days, spoilage organisms were isolated from all treatments.

Results: Two log CFU/g outgrowth of *L. monocytogenes* was observed in the control treatment after 28 days. Addition of 1.63% sodium lactate (60% w/w) resulted in 0.9±0.2 log CFU/g outgrowth. All acetate-based preservatives showed significantly better antimicrobial performance ($P<0.05$) compared to sodium lactate, with outgrowth of 0 to 0.3 log±0.3 log CFU/g after 28 days. Furthermore, isolation of spoilage organisms of each treatment after 28 days showed that different preservatives target different microorganisms with a shift towards mainly lactic acid bacteria in treatments containing acetates compared to a more diverse spoilage flora in the control and lactate samples.

Significance: The results of this study demonstrate the superior performance of acetate based antimicrobial solutions to control outgrowth of *L. monocytogenes* in smoked salmon at abusive temperatures storage conditions, and thereby assuring food safety of smoked salmon.

P1-248 Assessment of the Diversity of Bread Spoilage Fungi from Global Samples of Wheat Flour and Development of a Targeted Mold Cocktail for Shelf-Life Assessment

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Introduction: Each bakery faces unique challenges in terms of mold spoilage organisms. Out of the raw materials, flour is considered as one of the major contaminants. This knowledge will help to develop targeted mold cocktail for shelf life study.

Purpose: The purpose of this study is to elucidate the bread specific spoilage flora present in flours from regions across the globe and enable development of targeted mold cocktail for challenge studies.

Methods: 11 flour samples from 5 different regions (Middle East, India, North America, Latin America & Sweden) were collected. Standard white bread was inoculated with 1 g of the different flours in a sealed plastic bag and uninoculated bread was used as control. After inoculation, the breads were

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transferred into clean plastic bags and incubated at 30 °C until spoilage occurred. Individual colonies were isolated and identified through ITS sequencing and classical fungal strain typing.

Results: In total, 16 fungal species were isolated of which 15 could be identified through strain typing. The species identified were *Aspergillus flavus*, *Penicillium griseofulvum*, *Aspergillus candidus*, *Cladosporium cladosporioides*, *Penicillium spinulosum*, *Penicillium chrysogenum*, *Rhizopus arrhizus*, *Mucor circinelloides*, *Alternaria alternata*, *Penicillium atrosanguineum*, *Aspergillus candidus*, *Trichoderma citrinoviride* and *Talaromyces islandicus*. *P. griseofulvum* was found in flour from 3 different regions, while most species (11) were only identified in a single flour sample. All flour samples contained at least 2 different species while in some more than 5 different species were identified.

Significance: Flour is often regionally sourced, and its fungal population varies between regions. This study demonstrates that it is important to evaluate the specific source of spoilage organisms in bakeries when developing shelf life extending measures.

P1-249 Determination of Weak Organic Acid Resistance Kinetics of Prevalent Fungal Strains Found in Different Global Bakeries

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Introduction: Global diversity of mold spoilage strains is a major issue for bakeries to develop targeted shelf life using weak organic acid salts. Each bakery faces unique challenges in terms of spoilage organisms, setup of the facility, hygiene levels and product categories.

Purpose: This study focuses on the difference in weak organic acid resistance kinetics of different fungal strains commonly found in bakery environments.

Methods: White bread were prepared with different levels of weak acid preservatives (acetate and propionate). Spore suspensions with different concentrations (1-4 Log) of *Penicillium roqueforti*, *Cladosporium cladosporioides* and *Aspergillus* were inoculated on the bread's crust. Bread was stored in plastic bags and incubated at 25 °C until visible growth was observed or for 60 days.

Results: Out of the tested strains, *P. roqueforti* was the most resistant. The lowest inoculum level spoiled after 21 days, while higher concentrations (2-4 log) were fully molded within 13 days or less. In contrast, the lowest inoculation levels (1 log) of *C. cladosporoides* and *A. niger* did not spoil within the time of the experiment, while their higher inoculum levels (3 and 4 log) showed molding after 27 days and 35 days, respectively.

Significance: This study shows the impact of varying inoculum levels of different mold strains on resistance kinetics and highlights the importance of interspecific variation when assessing the efficacy of weak acid-based preservatives and shelf life.

P1-250 Developing Scientific Methods for Bread Shelf-Life Studies for Shelf-Life Extension Using Fermentate Solution

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Introduction: Shelf-life extension is top of mind for many bakeries, to maintain bread quality throughout supply chain and to minimize food waste. Preservation solutions need to be validated for shelf life before being implemented. Implementing a clean label preservation solution requires validated shelf life studies.

Purpose: To develop and assess different shelf life protocols and inoculation methods for bread shelf life estimation using fermentation based clean label preservation system.

Methods: Loaves of standard US white bread were prepared with either no preservatives, or with varying levels of Upgrade CR33 (1.8-2.1%). Bread dough was adjusted to target a final bread pH of 5.2. Regular shelf life and spot inoculation testing were compared. For regular shelf-life testing, uninoculated breads were used. For spot inoculation, standardized mold spore suspensions of (*Penicillium roqueforti, Aspergillus niger, Cladosporium cladosporoides)* were pipetted throughout the bread surface. All breads were placed in clear bags and stored at 25°C, monitoring daily for visible mold growth.

Results: Non-inoculated breads showed high variability in data, with many variables never showing visible mold before factors such as staling impacted results. Spot inoculation method showed consistent results, with negative control breads molding consistently by 5-7 days. Upgrade CR33 showed shelf-life extension of +83% and +100% at 1.8% and 2.1%, respectively, compared to no-preservatives treatment which gave 6 days of shelf life.

Significance: These results highlight the importance of inoculated studies for accurate validation of a preservation solution and substantiates the preservation efficacy of Upgrade CR33 solution.

P1-251 Multispectral Imaging in Combination with Machine Learning for the Microbiological Quality Assessment and Discrimination of Various Types of Mussels

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Introduction: Marketing products of higher quality, following sustainable practices resulting in higher profitability are issues of great concern in Aquaculture. Methods that are suitable for on-line, real-time seafood quality assessment which are also rapid and non-invasive could contribute to an upgrade of this sector.

Purpose: The investigation of Multispectral Imaging (MSI) in combination with regression and classification algorithms for the estimation of the microbiological quality and discrimination of mussels based on geographical origin and form.

Methods: Mussel samples (n=400) (fresh with and without the shell, frozen/thawed) of different species and different geographical origin (Greek and Spanish) were stored under various temperature conditions for specific time intervals. Total Aerobic Counts (TAC), and specific spoilage microorganisms' populations were determined while, MSI analysis (VideometerLab) was also conducted. PLS regression was used to assess the correlation between MSI and microbiological data. The dataset was split in training and test set while, the root mean squared error (RMSE) and coefficient of determination (R^2) were used as metrics for the evaluation of models' performance. Additionally, PLS-DA models were developed for the discrimination of samples based on geographical origin and form (fresh with and without the shell and frozen/thawed).

Results: Microbial counts ranged from 3.5 to 9.0 log CFU/g. PLS-R algorithm was efficient in predicting the microbial populations in fresh samples (R^2; 0,74, RMSE; 0.78), while the same model presented less satisfactory results in predicting the microbial populations in thawed samples. Moreover, 80% of samples were grouped correctly based on their origin, while the discrimination based on the different form was also successful as 91% of the samples were grouped into the correct category.

Significance: The combination of machine learning with MSI could be effectively used to estimate microbial population and ensure authenticity in mussels. This work has been funded by the project DiTECT (861915).

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P1-252 Effects of a Bacteriophage Cocktail Treatment on Spoilage Bacterial Growth in Catfish Fillet during Refrigerated Storage

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Introduction: Despite efforts to control specific spoilage organisms in fishery products, aquatic food spoilage still causes significant disease and economic burden.

Purpose: Catfish spoilage by mainly *Aeromonas* and *Pseudomonas* causes significant economic losses. Bacteriophages were used as a promising mitigation strategy to control the specific spoilage bacteria.

Methods: Bacteriophages were collected from Delaware aquaculture facility ponds and other surface water. The bacteriophages specific for *Aeromonas* and *Pseudomonas* were characterized by carrying out host specificity testing, including evaluating the effects of osmotic shock, various chemicals, different multiplicities of infection, and different pH and temperature ranges on the phage. We selected the fittest phage candidates and formulated them for phage therapy. Phage cocktails were evaluated for their efficiency in controlling the spoilage and total bacterial growth in challenged catfish fillets stored at 4°C. All data were evaluated in reducing bacterial populations among the different treatments using ANOVA for significance.

Results: Out of 18phages against forty-two bacterial strains tested, phage was 78% specific to the host bacterial species. Fourteen phages were specific to 21 bacterial strains from one species, two phages to two species, and others to four species. Most phages remained active up to 60°C, at pH range between 4 and 12, were not affected by osmotic shock treatment, and were stable in most chemicals and other stresses. We discovered a multiplicity of infection (MOI) of 5 was the best in this study. The phage cocktail-treated fillet had significantly slower bacterial growth than the single phage-treated and untreated fillet over a period of 120 hours.

Significance: The phage isolates had good stability in the different environments and significantly reduced bacterial growth in the fishery products. This makes it a good candidate for phage therapy applications to reduce fish spoilage bacteria in catfish products.

P1-253 Application of High Voltage Atmospheric Cold Plasma (HVACP) Technology to Decontaminate *Botrytis cinerea* Mold on Strawberries

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Undergraduate Student Award Entrant

Introduction: *Botrytis cinerea* is a common plant pathogenic mold in strawberries. It is a major cause of fruit spoilage and decreased shelf life. Annually, over 64% of fresh strawberries harvested in North America are wasted due to mold spoilage and are valued at approximately $1.4 billion. Over time, *B. cinerea* has become resistant to many fungicides and so there is a need to find an alternative method to decontaminate strawberries and increase their shelf lives.

Purpose: Decontaminate *Botrytis cinerea* from fresh strawberries with minimal quality change using a dielectric barrier discharge HVACP treatment.

Methods: 25 g of strawberries were spot inoculated with a load of 6 $\log_{10}$ CFU/g of *Botrytis cinerea* mold spores and stored at 4°C overnight. They were then exposed to indirect HVACP treatment with optimized conditions (90 kV, 5 minutes, air, 100% RH, 170W) and evaluated at 0hr, 4hr, and 24hr post-treatment storage times. Three samples in duplicate were plated for enumeration on Potato Dextrose Agar and viable mold counts were obtained after five days. Data sets were then analyzed using one-way ANOVA ($\alpha = 0.01$).

Results: The results showed an increasing log reduction of *B. cinerea* spores with increasing post-treatment storage time. A mean log reduction of 1.4, 2.1, and 3.0 CFU/g were obtained as the storage time increased from 0hr, 4hr, and 24hr, respectively. The HVACP treatment did not cause any significant changes in the strawberry firmness or color up to 24hr post-treatment storage.

Significance: This study demonstrates the potential use of HVACP treatment in air at room temperature to significantly reduce mold on fresh produce and increase their shelf-life without changing the physical characteristics. Additionally, HVACP is a low-energy, non-thermal process that may replace the current methods of fungicides and thermal treatments, further reducing water consumption and energy use.

P1-254 Prevalence of Fungi from Fresh Tomatoes and Their Control by Lemon Peel Essential Oil

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Introduction: Fungal contamination and harmful mycotoxins in fresh produce are deemed biological hazards according to the FSMA Produce Safety Rule. Chemical fungicidal compounds are frequently used to control postharvest fungal decay (Hadian et al., 2008). Synthetic fungicides cause several environmental and health problems due to teratogenic, carcinogenic effects and emergence of resistance against these fungicides (Tian et al., 2011). As a clean-label alternative, natural products such as citrus EOs can be an excellent alternative to synthetic fungicides.

Purpose: To evaluate the anti-fungal potential of natural EOs extracted from lemon peel and to study their effect on the quality of fresh tomatoes.

Methods: Fresh tomato samples (n=150) were collected from local grocery stores. EOs from the dried and fresh peels of lemon were obtained by Soxhlet apparatus using n-hexane and ethanol. Antifungal activity of EOs against Aspergillus and Penicillium spp was tested by "radial growth inhibition assay". Fourier Transform infrared spectroscopy (FT-IR) was performed to identify the chemical composition of EOs.

Results: *Aspergillus* (44%) and *Penicillium* spp. (33%) showed a high prevalence in tomatoes of different stores, followed by *Curvularia* (13%) and *Rhizopus* (10%) spp. EOs extracted by ethanol from powdered peels of lemon (PPL) gave 100% inhibition against *Penicillium* spp., whereas the hexane extracted EO from fresh lemon peels was also able to show 100% *Penicillium* growth inhibition. Antifungal effect of lemon peel EOs was significantly lower ($p < 0.05$) against *Aspergillus* spp., and maximum of 85.5% inhibition was recorded for EO of PPL extracted with ethanol.

Significance: The study suggests that the lemons peel EOs showed strong antifungal potential against fungi isolated from fresh tomatoes and can be used as a nature preservative to prevent fungal decay of fresh food produce.

P1-255 Application of Torreya Essential Oil for Food Safety and Shelf Life Extension

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Introduction: Essential oil of Torreya grandis Fort has a pleasant and mild floral/fresh/fruity aromas. Therefore, it is used for the flavoring of foods, such as chewing gums, sweets, teas, soft and energy drinks, milk products, as well as shampoos, soaps, shower gels, body lotions, and toothpastes. However, its antimicrobial property against foodborne pathogens and spoilage microorganisms has not been explored.

Purpose: This study was to investigate the antimicrobial activities of Torreya Essential oil (T-EO) *in vivo* and *in vitro*.

Methods: T-EO was extracted by hydrodistillation from the peel of Torreya grandis fort. The compounds in water phase (T-Liq) were also used for the comparison. T-EO alone or T-EO incorporated into chitosan films (T-EO-CHI film) was added into peptone water inoculated with *Escherichia coli* K12 (G-), *Listeria innocua* (G+) and the spoilage bread fungi. The reductions of these microorganisms were determined. The growth of fungi on bread exposed to T-EO or T-EO-CHI film and stored at room temperature was also monitored.

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Results: T-EO reduced *E. coli* populations from 8.2 to 5.5 log CFU/g, *Listeria* from 7.6 to 2.5 log CFU/g, and bread fungi from 5.5 to 1 log CFU/g. Addition of T-EO in chitosan film (T-EO-CHI film) achieved two-log greater reduction of both *E. coli* and *Listeria* than the chitosan film used alone. There were visible moldy spots on control bread samples stored at room temperature after one week, while T-EO or T-EO-CHI film treated samples had no such spots through three months. T-Liq did not show any antimicrobial activities against these microorganisms.

Significance: T-EO is a plant extract with mild pleasant odor. The results demonstrate that T-EO has great potential to be used as a natural antimicrobial agent in food for improving safety and shelf life of various foods, in addition to its food flavoring.

P1-256 The Antimicrobial Effectiveness of Gelatin Film Containing Oregano Essential Oil for Preservation of Blue Catfish (*Ictalurus furcatus)*

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✦ ***Undergraduate Student Award Entrant***

Introduction: While seafood is highly profitable, it is equally perishable due to bacterial spoilage. Gelatin coatings infused with oregano essential oil can be used on catfish to extend shelf-life.

Purpose: The purpose of this study was to determine the effectiveness of a gelatin film containing oregano essential oil for its antimicrobial properties as a method of preservation for catfish.

Methods: Fresh catfish fillets were purchased from a local fishery and separated into their respective treatment groups; control (C), gelatin (G), essential oil (EO), and gelatin with EO (GEO) in duplicates and at random. The respective samples were submerged in their respective treatments for 60 seconds and allowed to air dry for 10 minutes. Once dried, they were placed individually in a Ziplock bag and stored in refrigeration (4°C). Physical, chemical, and microbial analyses were conducted every four days for a period of 20 days.

Results: The Aerobic Plate Count (APC) displayed a significance between treatments C and GEO on day 8 with a mean of 5.47±0.35 log CFU/g and 4.03±0.54 log CFU/g, respectively. Psychrophilic displayed treatments EO (5.51±0.18 log CFU/g) and GEO (6.26± 0 log CFU/g) being significantly different from treatments C (7.86±0 log CFU/g) and G (8.14±0.06 log CFU/g) on day 12. Overall, EO and GEO resulted in a longer shelf-life compared to C and G, the latter groups shelf-life was 12 days, while EO and GEO had acceptable Psychrophilic counts at day 20 (4.26±0.77 log CFU/g and 6.28±0.10 log CFU/g). Similar results were also recorded for Enterobacteria, Yeast, and Mold.

Significance: Gelatin coating infused with oregano essential oil in addition to moisture active packaging increased the shelf-life of catfish.

P1-257 Enhancement of Fresh and Thawed Catfish Fillets Quality by the Application of a Gelatin Coating with Oregano Essential Oil Stored in a Moisture-Control Packaging

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❖ ***Developing Scientist Entrant***

Introduction: High perishability of seafood creates the need to find methods to extend its shelf life. Moisture control packaging and essential oils could assist in improving shelf life by controlling moisture and inhibiting bacterial growth.

Purpose: The purpose of this study was to evaluate the effect of gelatin combined with oregano essential oil using a moisture control packaging to extend the shelf life of fresh and previously frozen catfish fillets.

Methods: Fresh catfish fillets were treated with gelatin (G) and gelatin infused with essential oil (GEO), half were place in moisture control trays (GM and GEOM). Then half of each group was stored in refrigeration (4°C), the other half were frozen and thawed within 5 weeks for frozen storage (previously frozen). Physical/chemical and microbial activity were analyzed every four days for a period of 16 days for fresh and previously frozen samples.

Results: Initial Aerobic Plate Count (APC) of fresh GEO and GEOM (3.28±0.28 and 3.29±0.41 log CFU/g) showed lower counts compared to G and GM (3.96±0.5 and 4.16±0.08 log CFU/g), similar trend was observed for the previously frozen groups. Psychrophilic counts for G and GM surpassed the acceptable limit at day 12 (7.24±0.8 and 7.79±0.18 log CFU/g), while GEO and GEOM remained below the limit at day 16 (6.71±0.93 and 6.74±1.25 log CFU/g). Similar trend was observed for previously frozen samples with a slight increase. Fat oxidation of fresh GEO and GEOM showed lower mg MDA/Kg than G and GM, which at day 16 were 0.39±0.06, 0.36±0.11, 1.28±0.39, and 1.32±0.30, respectively. Similar trend with no increase was observed for previously frozen samples. The statistical method used was analysis of variance (ANOVA), followed by the Tukey's test ($\alpha = 0.05$).

Significance: Adding EO to a gelatin coating enhanced the shelf-life of catfish fillets, however no synergistic effect was observed with moisture control. Furthermore, the benefit of the EO was observed after a frozen and thawing cycle.

P1-258 Sustainable and Biodegradable Chitin Films from Waste Crab Shells for Food Packaging

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Introduction: Seafood industries are facing the problem of effectively dealing with seafood bioproducts and waste such as crab shells. At the same time, traditional food packaging materials such as polyethylene (PE) are becoming the major source of microplastics, hampering the wellness of people and the environment. When traditional biopolymer films are poor in barrier and technical performance, chitin nanofiber (CNF) as the reinforcement filler showed potential to tackle these problems.

Purpose: To effectively prepare CNF and develop CS/CNF film for food packaging.

Methods: Fresh crab shells were collected and washed, followed by drying and grinding into fine powder. 3% HCl and 5% NaOH were applied to eliminate protein and minerals in the shell. Ultrasonication was applied to disintegrate chitin into nanofiber without any other chemicals. The parameter was set as 700 W, 100% amplitude and 8 min. After that, chitin was collected by centrifugation at 2000 rpm. 1 g chitosan was dissolved in 1% acetic acid solution with varied CBNF concentration, 10%, 20% and 30%. Casting method was used for film formation at 30°C oven heating. 0.025% curcumin was added for functional purposes. Pure chitosan film was prepared as comparison and the storage condition is 25 °C with 50% RH.

Results: Chitin nanofiber was successfully prepared, which are supported by FTIR, XRD, TGA and AFM result. Barrier properties including oxygen/water vapor UV-blocking were measured to showed the slight decrease in WVTR and reinfoced UV blocking ability. TGA resulted showed the increase in thermal retardant with CNF addition. Antioxidative ability (DPPH) was significantly increased for CS/CNF/Cur film. A 10% increase was found for 30% CS/CNF film in elongation at break.

Significance: Chitin nanofiber was successfully prepared and showed increased barrier and mechanical properties for chitosan film.

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P1-259 The Effect of Xanthan Gum on the Efficacy of Laminated Antimicrobial Films to Inhibit Foodborne Pathogens Associated with Beef Products

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Introduction: Laminated antimicrobial films (LAFs) have been developed by coating a mixture of pullulan, xanthan gum (XG), and lauric arginate (LAE), a broad-spectrum, cationic surfactant, onto the polyethylene side of ethylene vinyl alcohol (EVOH) films. The resulting LAFs can improve the microbial quality and safety of meat and poultry products.

Purpose: When combined with anionic polysaccharides such as XG, LAE can form soluble or insoluble complexes, thereby affecting its antimicrobial activity. This study evaluated the impact of XG in the formulation of LAFs and the antimicrobial activity of LAE through a series of experiments.

Methods: LAFs made with 10.6% (135g/L) pullulan, 2.5% LAE (30-31g/L), and 0 or 0.2% (0-2.52 g/L) XG were assessed for antimicrobial activity using plate overlay assays containing >7 $\log_{10}$CFU/ml of a panel of pathogenic and nonpathogenic *E. coli* and *Listeria spp*. (n=54). In a subsequent challenge study, *E. coli* and *Listeria* spp. were experimentally inoculated onto 25 cm^2 of raw beef and ready-to-eat (RTE) roast beef to obtain populations of ~8 $\log_{10}$CFU/cm^2, treated with various formulations of the LAFs, and remaining microbial populations determined up to 28 days at 6˚C (n=252).

Results: Results indicated that LAFs containing 0% XG exhibited significantly higher zones of inhibition (P < 0.05) against the pathogenic organisms than LAFs made with 0.2% XG. While LAFs containing 0 and 0.2% XG reduced *E. coli* and *Listeria* spp. on raw beef and roast beef, as compared to EVOH controls, reductions between 0 and 2.5% XG were not seen across all treatments or days.

Significance: This study demonstrates LAFs containing 0% XG exhibited increased antimicrobial activity against microorganisms in plate overlay assays compared to 0.2%, but this activity was not observed throughout the challenge study. The results suggest that the antimicrobial activity of LAFs may be affected by meat matrices (ex. protein, fat), and thus may impact LAE migration.

P1-260 Development and Evaluation of Chitosan-Pullulan Films Containing Lauric Arginate (LAE) to Inhibit Microorganisms Associated with Raw and Ready-to-Eat Meat Products

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Introduction: Food spoilage organisms and foodborne pathogens associated with raw and ready-to-eat (RTE) foods are major contributors to food waste and foodborne illness, raising economic and health concerns among consumers and food manufacturers alike.

Purpose: The objective of this study was to evaluate the antimicrobial effectiveness of laminated antimicrobial films (LAFs) containing chitosan, pullulan, and lauric arginate (LAE) when applied to muscle foods.

Methods: Antimicrobial activity of the LAFs and control films was assessed through plate overlay assays, along with a shelf-life and challenge study. The shelf-life study evaluated LAFs against aerobic (AC) and lactic acid bacteria (LAB) associated with store-bought raw and RTE roast beef. The challenge study evaluated LAFs against ~8 to 9 $\log_{10}$ CFU/ml cocktails of *Listeria seeligeri*, *Listeria innocua*, *E. coli* O157:H12*,* and *E. coli* O150:H8 experimentally inoculated onto raw and RTE roast beef.

Results: Plate overlay assays determined that LAFs containing 0.5% LAE (0.5 mg/mL) displayed antimicrobial activity against a panel of nonpathogenic and pathogenic strains of *Listeria* spp. and *Escherichia coli*. The shelf-life study demonstrated that LAFs containing 0.5% LAE applied to raw beef reduced AC and LAB by 7 $\log_{10}$ CFU/cm^2 when compared to control films. However, no significant differences (P > 0.05) were observed in the growth of AC or LAB between film treatments applied to RTE roast beef. The challenge study determined that after 28 days of storage at 4°C, LAFs containing 0.5% LAE reduced *Listeria* spp. and *E. coli* by 2.77 and 2.93 $\log_{10}$ CFU/cm^2, respectively, on raw beef. Reductions of 3.96 and 4.29 $\log_{10}$ CFU/cm^2 of *Listeria* spp. and *E. coli* were observed, respectively, on RTE roast beef.

Significance: The results of this study demonstrate the potential use of LAFs containing chitosan and LAE in extending the shelf-life and reducing potential pathogens associated with raw and RTE muscle foods.

P1-261 Polyvinyl Alcohol/High Amylose Starch-Based Active Composite Film with Montmorillonite and Anthocyanin for Smart Monitoring of Meat Freshness

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Introduction: Due to the food safety and freshness concerns of consumers, besides the increased awareness towards environmental and health-related issues of petroleum-based plastics, active and intelligent packaging systems based on biodegradable polymers were developed to mitigate side effects of non-biodegradable plastics and meet consumers' requirements to maintain and monitor food quality and safety.

Purpose*:* The aim was to develop active and smart packaging film based on polyvinyl alcohol (PVA), high amylose starch (HAS), reinforced with montmorillonite (MMT K10) and anthocyanin (ANT) extracted from hibiscus.

Methods*:* The blend film was prepared by solvent-casting method at ratio of 3:1 (PVA:HAS) using different concentrations of MMT (1, 2, 5, and 7% w/w of polymers). ANT was added to optimal film with MMT to increase antimicrobial, antioxidant and pH-sensing properties. Fabricated films' structural, morphological, barrier, mechanical and optical properties were studied.

Results*:* Addition of MMT significantly (*P*< 0.05) increased tensile strength (n=3 for each film), thermal stability (n=3 for each film), water-resistance (n=3 for each film), color parameters (n=3 for each film), and reduced WVP of films (n=3 for each film). The films' antioxidant, antibacterial and pH-responsive properties were enhanced upon ANT addition. The structural and morphological studies confirmed interactions of film ingredients and their distribution into film matrix. MTT assay inferred the non-toxicity of films' components against normal WI-38 cell lines. The smart film produced distinguishable colors in response to different spoilage conditions of stored chicken at different storage temperatures (25, 7 and -20 °C).

Significance*:* To the best of our knowledge, this is the first report on the fabrication of a smart and active film based on PVA and high amylose starch blend enhanced with MMT and activated with ANT. Results provided evidence for the potential applicability of the smart film as a promising safe candidate for monitoring of meat spoilage at different storage conditions.

P1-262 Evaluation of Invisishield™ Technology, a Chlorine Dioxide Based Packaging System, to Inactivate Hepatitis A Virus on Blueberries Under Frozen Conditions

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Introduction: Controlled release chlorine dioxide (ClO_2) is a promising antimicrobial that has yet to be evaluated in frozen foods.

Purpose: To evaluate a novel ClO_2-based antimicrobial packaging system (InvisiShield™) for its efficacy at inactivation of hepatitis A virus (HAV) on individual quick frozen (IQF) blueberries.

Methods: Fresh blueberries purchased from commercial sources were decontaminated and inoculated with ~6.0 $\log_{10}$ HAV genome equivalent copies (GEC, cell culture lysate) per berry sample group. After overnight refrigeration, blueberries were IQF-mock frozen using liquid nitrogen and placed into

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glass jars with an InvisiShield™ strip suspended inside. Four treatment doses (1.0, 2.0, 3.0, and 4.0g-ClO^2/lb) and a control (no strip) were evaluated. The sealed jars were stored at -15°C (±2°C) for 15 and 30 days. At each time point, including a Time 0 positive and negative control, the product was collected and processed for virus concentration using the sequential steps of elution, polyethylene glycol precipitation, and chloroform-butanol extraction. Concentrates were pre-treated with RNase, extracted for RNA isolation, and quantified by RT-qPCR. GEC was determined by comparison to standard curve and $\log_{10}$ reductions (LR) were calculated by subtraction of GEC in treatment from that in the Time 0 positive control. Trials were performed in triplicate.

Results: No statistically significant difference in LR was observed when comparing exposure time at any one ClO_2 dose. Therefore, all results were combined to evaluate differences between doses. LR in HAV GEC was 2.8±0.6, 3.2±0.5, 3.2±0.5, and 3.3±0.4 at 1.0, 2.0, 3.0, and 4.0-g/lb, respectively, the latter of which corresponded to complete elimination of RT-qPCR signal.

Significance: At doses $\geq$2.0g/lb, this novel ClO_2-based antimicrobial packaging system produced >3.0 LR in HAV GEC on IQF blueberries after 15 days of frozen storage. Future studies exploring shorter exposure times are warranted. The technology shows promise for inactivating enteric viruses on IQF produce items.

P1-263 Effects of Helium Gas Utilization in Modified Atmosphere Packaging (MAP) on Beef Quality

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Developing Scientist Entrant

Introduction: Meat spoilage is an economic burden and contributes to the development of off-odors, flavors, and changes in product appearance. This study evaluated the effects of helium gas utilization in MAP (modified atmosphere packaging) on shelf life and reduction of Biotype I *E. coli* microorganisms on beef surfaces.

Purpose: To determine if including helium gas in MAP impacts color, shelf-life, and microbiological reductions of *E. coli* surrogates on steak surfaces.

Methods: USDA Choice, boneless strip loin steaks were allocated to one of three MAP packaging treatments: (1) control (60-80% O_2 and 20–30% CO_2), (2) high helium (60% O_2 , 10% CO_2, 30% He), (3) low helium (80% O_2 , 10% CO_2, 10% He). Steaks were analyzed on day 0, 3, and 5. Top and bottom lean surfaces of steaks (n = 12/treatment) were inoculated with five non-pathogenic *E. coli* Biotype I surrogate strains. Pre-treatment and post-treatment samples were prepared and plated using appropriate serial dilutions and plated on 3M™ Petrifilm™ *E. coli*/Coliform Rapid Count Plates. For color assessement, steaks (n = 12/treatment) were held for 0, 3, and 5 days in a simulated retail display case. Visual and objective assessments of lean and fat color were conducted before and after a 30-min bloom time.

Results: Microbial growth, ranging from 0.4 to -0.2 log/cm^2, occurred on both top and bottom surfaces for both high and low helium treatments. Panelists ratings for lean color, ranging from 1.7 to 2.5, and lean discoloration, ranging from 0.0 to 0.1, were not different (P > 0.05) among treatments. There were no identified trends among treatments for objective lean color scores for CIE L* (lightness), a* (redness), and b* (yellowness) color space values.

Significance: Helium gas was ineffective in reducing *E. coli* Biotype I microorganisms on beef surfaces, and there was little impact on meat color.

P1-264 Performance of a Novel Multifunctional Carboxymethyl Cellulose Film Incorporated with Lemon Essential Oil Nanocapsules in Active Packaging of Tomato and Baby Spinach Leaves

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Introduction: Carboxymethyl cellulose (CMC) is an attractive cellulose-derived polymer for its biocompatibility, biodegradability, low cost, non-toxicity, and favorable organoleptic properties. However, the adoption of CMC-based films in food packaging sectors is restricted in view of their low tensile (TS)/modulus and poor water retention quality.

Purpose: This is the first report of a new active CMC-based film incorporated with a cost-effective bioactive ingredient that significantly improved the film's biological, barrier, hydration, mechanical, and thermal properties, and maintained the fresh-keeping qualities of fresh produce under abusive storage temperatures.

Methods: Lemon essential oil (LEO) extracted from lemon peels by hydrodistillation was encapsulated using maltodextrin (MD; 10% w/v) as the wall material. Sorbitol plasticized active films were prepared using a solution casting technique by either incorporating free LEO or MD-LEO micro/nanocapsules (0.4% w/w of the polymer) into CMC (2% w/v) matrix. Control (neat) and active films were evaluated for their microstructure and optical, chemical, thermal, biological, and mechanical properties.

Results: The stable freeze-dried nanocapsules (80–300 nm) exhibited cytotoxic, strong antioxidant capacity, potent antimicrobial activity against important foodborne pathogens, significantly (P<0.05) reduced the film's water vapor permeability by 37.5%, and increased its thermal stability by 15-25°C. The CMC-MD-LEO transparent film completely degraded (96.84%) after 2 weeks of soil burial, demonstrated double the TS value (30.14 MPa) of that with free LEO, and contained half the moisture of the neat film. Wrapping experiments revealed that the novel active film improved textural/visual attributes and microbiological quality of cherry tomato samples, in addition to inhibiting the growth of *E. coli* O157:H7 populations on baby spinach leaves after storage at ambient temperature for 6 days.

Significance: Results of this work are encouraging and it is possible that the multifunctional CMC-MD-LEO film used for edible active packaging may be adapted to other pertinent food systems in the future, or present a promising candidate in commercial food applications.

P1-265 Reduction of Pathogen Surrogate Bacteria Using an Aqueous Ozone Intervention, on Diced Fruits and Vegetables

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Introduction: Due to recent Shiga-Toxin Producing *E. coli* (STEC) and *Salmonella* outbreaks associated with produce, the produce industry is continuously evaluating intervention strategies to mitigate the risks of pathogens that can cause foodborne illness.

Purpose: To determine the antimicrobial efficacy of BioSafe®-aqueous-ozone intervention applied to frozen and fresh fruits and vegetables challenged with *E. coli* surrogates.

Methods: A cocktail of five non-pathogenic strains of *E. coli* (ATCC-BAA 1427, 1428, 1429, 1430, and 1431) that are suitable surrogates for *Salmonella* and *E. coli* O157:H7 was used to inoculate the surface of blueberries, carrots, lettuce, spinach, and broccoli at 5 LogCFU/g. The ozonated-water treatment was applied at an oxidation-reduction-potential (ORP)> 850mV. For each fruit and vegetable, 25g samples were treated using a spray bottle with the surrogates cocktail and 20 minutes were allowed for cell-attachment. From each product, 10 uninoculated samples were tested for aerobic counts (AC) to enumerate natural microbiota and 20 inoculated samples were collected before and after treatment spray to enumerate *E. coli* Counts (EC) using the Tempo®-System. A t-test was performed using R-software(Version 4.04).

Results: Natural microbiota was between 2.12, and 5.58 (LogCFU/g). *E. coli* counts (LogCFU/g) were significantly reduced (P<0.001) between attachment and after treatment on average by 1.32, 2.08, and 1.58 LogCFU/g on blueberries, lettuce, and spinach, respectively. *E. coli* counts (LogCFU/g) on broccoli and carrots were not significantly reduced (P>0.05) comparing attachment and after-treatment samples.

Significance: Aqueous ozone intervention was shown effective in our challenge trial for blueberries, lettuce and spinach indicating it can be applied in industrial scale as an intervention to reduce potential contamination with pathogens. It is reasonable to conclude that the spray-treatment is effective in reducing *Salmonella* spp. and *E. coli* in blueberries, lettuce, and spinach, additional optimization of the ozone treatment is needed for the use in other products.

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P2-01 Safety Assessment of Raw and Extruded Canine Diets and Antimicrobial Susceptibility Testing of the Isolated Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: The use of raw meat-based diets (RMBDs) is a growing trend among pet owners. RMBDs can be a source of pathogenic bacteria that might represent a public health risk, particularly for vulnerable populations in healthcare.

Purpose: To identify foodborne pathogens in extruded and raw canine diets, canine fecal samples, and to determine the antimicrobial susceptibility of the isolates.

Methods: Sixty-six canine diets (RMBD=42; extruded=24) and fifty-five fecal samples (raw-fed dogs=33; extruded-fed dogs=22) were analyzed for the presence of *Salmonella* spp., *Listeria monocytogenes*, and *Campylobacter jejuni* by modified BAM methods. Bacterial species were identified by biochemical and PCR tests. *Salmonella* and *Listeria* isolates were sequenced in Illumina platforms. Antimicrobial susceptibility was determined for *Salmonella* (n=19) and *Campylobacter* (n=2) through the Kirby-Bauer disk diffusion method against 13 antibiotics.

Results: *Salmonella* and *L. monocytogenes* were only detected in RMBDs (35.7%, 15/42). *Salmonella* spp. was isolated from 26.2% (n=11) and *L. monocytogenes* from 19% (n=8) samples. *Salmonella*, *L. monocytogenes* and *C. jejuni* were identified in 33.3% (11/33) of fecal samples from RMBD-fed dogs. *Salmonella* spp. was detected in 24.2% (8/33) of the samples, *L. monocytogenes* from 3% (1/33) and *C. jejuni* from 6% (2/33) samples. No pathogens were detected from diets or fecal samples from extruded-fed dogs. *Salmonella* Infantis was the most frequent pathogen isolated (89.5%, 17/19). *Listeria welshimeri* (n=3) was identified in food samples whereas *Listeria inoccua* (n=6) from food and fecal samples. *Salmonella* isolates (n=19) showed a high frequency of resistance to nalidixic acid (78.9%, 15/19), ampicillin (68.4%, 13/19), and chloramphenicol (52.6%, 10/19). Nalidixic acid-ceftiofur-ceftriaxone-amoxicillin/clavulanic acid-ampicillin was the predominant resistance pattern (21%, 4/19). *C. jejuni* isolates showed 100% (2/2) resistance for ciprofloxacin, tetracycline, and gentamicin.

Significance: Our findings demonstrate the need to work on pet food safety strategies such as monitoring the presence of pathogens in RMBDs and RMBD-fed dogs, and survey their antimicrobial susceptibilities.

P2-02 Revisiting Spray Drying Technology for Co-Encapsulation of Probiotics and Phytochemicals as Alternative to Antibiotics in Livestock Feed

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Introduction: Antibiotics have been widely used in livestock feeds for decades, to prevent animals from diseases and improve production performance, while the abuse-resulted increasing emergence of antibiotic-resistant bacteria seriously poses dangers to human health and ecological equilibrium.

Purpose: Developing alternatives to antibiotics is an *urgent* need for poultry industries.

Methods: Relevant studies in recent five years have been searched in PubMed and Web of Science databases using “livestock”, “alternative antibiotic”, “probiotics” and “encapsulation” as keywords solely or in combination.

Results: Probiotics, as the superior rebuilders of gut microbial community, are the ideal candidate, inspired by the action mechanism of antibiotics related to killing harmful bacteria. Natural phytochemicals, including polyphenols and essential oil, could strengthen the immunity of animals to achieve the same effect as antibiotics. Excitingly, the co-microencapsulation of the two could exert synergistic health benefits and improve the stabilization of mixtures, compared to individual components. Spray drying is an old-fashioned but dominantly applied method of microencapsulation with the outstanding advantage of easy translation from laboratorial to industrial scale. Further, pre-embedding the contents before feeding using coprecipitation, emulsification and so on is promising to reduce the activity loss of heat-sensitive probiotics and phytochemicals during the spray drying process. Benefiting from the high availability and biocompatibility, natural polysaccharides are suitable embedding material for oral delivery. Besides, polysaccharides are only metabolized by the microbiota, achieving the targeted release of entrapped active ingredients to the colon, which is pretty crucial for probiotics to survive in the harsh environment of the upper gastrointestinal tract to accumulate in sufficient amounts in the large intestine.

Significance: The review systematically summarizes the most recent findings involving probiotics and phytochemicals as alternative antibiotics from effectiveness to co-packaging using spray drying, laying the groundwork for livestock feed production.

P2-03 Qualitative Application of Immuno-Magnetic Reduction as a Biotoxin Detection Technology for Animal and Pet Food Safety

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Introduction: Biotoxins such as mycotoxins, microtoxins, and phytotoxins are harmful substances produced in living cells or organisms. The novel techniques such as immunomagnetic reduction (IMR) can detect ultralow concentrations of biotoxin through the use of antibody-functionalized magnetic nanoparticles dispersed in an aqueous solution. Thus, it may be useful technology for biotoxin detection in animal and pet food.

Purpose: We shed light on using IMR as a biotoxin-detection technology for animal and pet food safety.

Methods: A SQUID (superconducting quantum interference device) is a very sensitive magnetometer that can measure extremely subtle magnetic fields. We developed SQUID-based alternating current (AC) magnetic susceptometer (XacPro-S, MagQu) to determine time-dependent AC magnetic susceptibility, and the association between the magnetic nanoparticles and target biotoxin molecules. The magnetic nanoparticles were dextran-coated Fe_3O_4 particles (MF-DEX-0060, MagQu). The biotoxin detecting reagent contained magnetic nanoparticles functionalized with monoclonal antibodies against the target biotoxin.

Results: Our data showed that IMR assay can rapidly detect ultralow concentrations of biotoxin such as mycotoxin and microtoxins in animal and pet foods. From immunohistochemical staining and western blotting evidences, we confirmed the validity of the IMR data.

Significance: We suggested that IMR can be a non-destructive technology that helps to rapidly assess biotoxin molecules in animal and pet foods.

P2-04 Detection of *Salmonella* spp. in 375 g Test Portions of Animal Feed and Pet Food Using a PCR Kit and a Chromogenic Medium

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Introduction: Animal feed and pet food remain challenging matrices for the detection of Salmonella due to the high level of background flora.

Purpose: This study evaluates the suitability of a new unique enrichment protocol to improve the detection of Salmonella in animal feed and pet food by both a PCR kit and a chromogenic medium specific for the detection of Salmonella.

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Methods: Sixty-two 375 g samples, shared among raw material, pet food and animal feed types and half of them being positive, were diluted 1/6 in Buffered Peptone Water supplemented with a Salmonella enrichment supplement and prewarmed at 37±1°C for 18-24 hr. For PCR detection, the iQ-Check Salmonella II kit was used (Easy I lysis protocol with or without free DNA removal treatment). For the detection on a chromogenic media, 10 µl of the enriched samples were streaked on a RAPID'Salmonella plate. Confirmation was carried out using conventional tests described in the ISO 6579-1 reference method. All samples were tested in parallel with the ISO 6579-1 reference method.

Results: Using PCR detection, 25 samples were found positive with both the reference method and the PCR method (positive agreement), 31 samples were found negative with both methods (negative agreement), 2 samples were found positive with the PCR method while found negative with the reference method (positive deviation) and 4 samples were found negative with the PCR detection while detected positive with the reference method (negative deviation). Using the detection on chromogenic media, 26 positive and 31 negative agreements were found, as well as 2 positive and 3 negative deviations.

Significance: The results of this study confirmed that the newly developed enrichment protocol allows the detection of *Salmonella* in 375 g samples of animal feed and pet food with performance fitting with expectations included in the ISO 16140-2 standard.

P2-05 Assessing Existing Food Safety Knowledge, Behaviors and Resource Needs for Growers and Supervisors Due to COVID-19

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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 produce. Training focuses on personnel who contact and/or supervise covered produce and/or food-contact surfaces. In addition to general health and hygiene, task-specific training is critical as workers are a known potential source of foodborne-pathogen contamination when working without knowledge of risks and mitigation strategies.

Purpose: A survey was administered to examine how COVID may have changed the way food safety training experience, knowledge, and practices among farm workers in Maryland.

Methods: The App2Train survey was developed by the project team to assess food safety knowledge and training among farm workers, and delivered by online survey form. The survey was sent via snowball sampling through the Maryland Food Safety Network in 2021 and had 65 respondents, of which 34 were owners or co-owners of the farm. Descriptive analyses were conducted to identify areas of need for resources or improvement in training practices around food safety.

Results: When asked about barriers to training in food safety, 58% of farm owners reported the training materials were not specific to activities on their farm, 83% reported issues with finding time to conduct training sessions, and 88% reported barriers with expenses to comply with training. Among all respondents, only 34% of survey participants believed they have enough awareness and information about on-farm food safety practices, although 90% reported that issues related to food safety, particularly foodborne diseases, were serious concerns for them.

Significance: Common themes for barriers to administering training for food safety practices were not having enough time or funds to administer proper training sessions, despite acknowledgement that food safety issues were a serious concern.

P2-06 The Impact of the COVID-19 Pandemic on Handwashing Behaviors during Breakfast Meal Preparation: Qualitative Analysis of Interview Findings

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Introduction: During the COVID-19 pandemic, government and public health agencies emphasized the importance of frequent and effective handwashing in reducing the transmission of the COVID-19 virus, but little is known about how these messages and the pandemic itself influenced self-reported handwashing behaviors during meal preparation.

Purpose: This study investigated if the COVID-19 pandemic and government handwashing recommendations influenced consumer handwashing behaviors (self-reported) during meal preparation.

Methods: A sample of 251 participants was recruited from various communities in North Carolina. Participants were asked to prepare a breakfast meal in a consumer-style kitchen. After meal preparation, researchers conducted semi-structured interviews with each participant. Interview questions included questions about handwashing recommendations since the start of the COVID-19 pandemic; how participants responded to these recommendations; and how handwashing habits changed during the course of the pandemic. The interviews were recorded, transcribed, and analyzed using Nvivo software.

Results: Eighty-seven percent of participants reported having heard government recommendations for handwashing since the start of the COVID-19 pandemic. A majority of participants (62%) reported increasing handwashing frequency; 34% reported that their handwashing habits stayed the same; and 4% reported an initial increase, followed by a change back to 'normal' practices. Qualitative analysis identified several reasons behind these behaviors including greater protection from potential "germs"; confidence in existing handwashing habits; and increased use of hand sanitizer. Additionally, approximately 10% of participants distinguished between handwashing during meal preparation and 'outside of the kitchen'.

Significance: These results improve our understanding of how consumers internalize public health recommendations particularly with respect to food handling behaviors and suggest that food safety messages should address consumer perceptions and confidence in order to increase the personal relevance and effectiveness of food safety messages.

P2-07 Assessment of the Effectiveness of a Piloted Online Delivery of Current Good Manufacturing Practices for Small Food Processors in Iowa

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Introduction: Implementing best practices for compliance with Current Good Manufacturing Practices (cGMPs) is critical for a small food processor, primarily because it is a prerequisite program before gaining their license.

Purpose: This study assessed participants' attitudes, perceptions, intentions, and behavior toward food safety practices using the four constructs from the Theory of Planned Behavior.

Methods: Thirty-two participants enrolled and completed an online pilot module on cGMPs. The effectiveness of the online module was measured by examining participants in pre-and post-test. Descriptive statistics (frequency, percentage, mean, and standard deviation) and Wilcoxon signed-ranks tests were used for the analysis. The effect sizes for the statistically significant results were large($r = 0.64$ and $r = 0.60$); however, a large number of statistical tests and the more conservative alpha level ($p = 0.001$) resulted in a greater probability of a Type II error rate. Thus, the results of this study should be interpreted with caution.

Results: Program participants had been preparing and selling foods for an average of 6 years (SD=7.9; Range: 0-35 years). Participants had a wide range of employees (0-200; M=54.6; SD=60.7). Pre and post-test were matched for the analysis. Wilcoxon signed rank was used. Statistically significant changes were observed from the pretest (Mdn=3.0) to the post-test (Mdn=4.0) on the perception of sufficient food safety implementation ($z=-3.606$; $p<0.001$; $r=0.64$); from the pretest (Mdn=3.0) to post-test (Mdn=4.0) on the perception of control over scheduling how often the facility's waste system was moni-

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tored (z=-3.419; p=0.001; r=0.60); and pretest (Mdn=3.0) to post-test (Mdn=4.0) on the perception of knowledge about appropriate food storage temperature (z=-3.419; p=0.001; r=0.60). Prochaska and DiClemente's Stages of Change Model determined program participants' intention to sell foods produced in their facility. There was no significant change from pre to post.

Significance: These findings reinforce participants' positive practices of food safety actions on the food production floor.

P2-08 Increasing Accessibility of Food Safety Education through Remote Learning

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Introduction: Produce safety trainings programs are designed to educate farmers on practices to help identify and reduce risks of foodborne illness. Trainings are typically in-person and instructor-led, which costs time and money; barriers which prevent beginning and disadvantaged farmers from attending.

Purpose: To address this inequity, the University of Florida (UF) in collaboration with Florida Agricultural and Mechanical University (FAMU) developed digestible, remote learning produce food safety videos that are accessible to small, beginning, and minority farmers.

Methods: Produce Safety Alliance (PSA) grower trainings were offered remotely by UF and FAMU to train students and farmers representing the target audiences. Results from previously conducted focus groups, workshop evaluations, and surveys were used to guide the development of short "Produce Safety in Minutes" (PSM) videos, instructor-led recorded webinars, and discussion-based recorded case studies. Trained students and farmers were used as advisory board members for review of the PSM video series and participated in instructor-led webinars.

Results: Six students and 19 farmers have successfully participated and received certificates in the remote PSA Trainings. Produce Safety resources including PSM videos (18), instructor-led webinars (8), and case-studies (4), have been recorded. Resources targeted topics such as handwashing, taking a water sample, cleaning and sanitizing, compost handling, etc. The advisory board review indicated the resources created increased their knowledge of the topic, would be used as a training tool on their operation, and is representative of the diverse groups of people working in the produce industry.

Significance: Farmers and students who participated in the PSA trainings are more prepared for Produce Safety Rule Inspection or to pursue a career in the produce industry. Accessible remote videos improve convenience of produce safety information to small, beginning, and minority farmers where traditional training settings require barriers to entry.

P2-09 Knowledge Has Increased but Some Concepts Remain Challenging: Key Learnings from the Western Region Food Safety Trainings

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Introduction: Since 2017, members of the Western Regional Center to Enhance Food Safety have been training regional stakeholders and assessing their pre and post-training knowledge following Produce Safety Alliance (PSA) and Food Safety Preventive Controls Alliance (FSPCA) trainings. Combining regional training data with the Food Safety Resource Clearinghouse resource search data can provide information on the current educational impact and key resource needs in the region.

Purpose: Evaluate the impact of food safety trainings and identify key resources searched by the Western Region (WR).

Methods: Standardized questionnaires were used in 39 PSA (25 questions) and 5 FSPCA preventive controls for human food trainings (15 questions) to assess pre/post-training participant knowledge. Clearinghouse activity was tracked with Google Analytics from September 2021 to August 2022.

Results: Average PSA pre-training (PreT) and post-training (PostT) scores were 16.07/25 (n=529 tests) and 19.60/25 (n=499), respectively. For FSPCA courses, average PreT (n=44) and PostT (n=44) were 5.16/15 and 7.34/15, respectively. Knowledge gain was similar for both trainings (~14%). Participants frequently answered questions incorrectly related to wildlife (200/499 post-tests; 40%), chemical hazards (39%), and recordkeeping (39%). In FSPCA trainings, answers to bacterial hazards (34/44 post-tests; 77%), critical limits (75%), and hazard evaluation (70%) questions were most often incorrect. Of the 5,124 WR visits to the Clearinghouse, wildlife was the most often searched term (10 unique searches), while sanitation procedures, TAN and manure had the lowest rates of users finding resources per search. The FSPCA Food Safety Plan Template was the most viewed resource.

Significance: The results show that trainings in the WR are effective at increasing knowledge in produce safety and preventive controls practices. There seems to be a trend between concepts that are often identified incorrectly and the Clearinghouse resource searches and gaps. These data can be used to develop new resources and improve training activities for less understood concepts.

P2-10 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 farmers, socially disadvantaged farmers, small food processors, and small fruit and vegetable wholesale merchants.

Purpose: The Southern Center for FSMA Training (SC) 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 2021-September 2022 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.

Results: Forty-one PSA (567 participants) and 2 FSPCA (27 participants) courses were held across 11 and 2 states/territories, respectively. PSA post-test scores (22.25/25; n=317) were significantly higher than pre-test scores (16.35/25; $t = 10.15$, $p < 0.01$), indicating a significant increase in knowledge. Of 13 practices included in the PSA surveys (n=46-58) the most adopted practices were implementing new or different practices for monitoring on-farm facilities (59.26%; 32/54) and implementing new or different methods for cleaning or sanitizing food contact surfaces (58.69%; 27/36). Of seven practices included in the PCQI behavior change surveys (n=41) the most frequent behavior change was fine-tuning existing food safety plans (21.95%).

Significance: Members of the SC have continued to create FSMA specific resources for growers and processors. The Lead Regional Coordination Center operates to promote produce safety, communication, and collaboration between the four regional centers at a national level.

P2-11 Hands-on Training on Food Safety Practices: Understanding the Hmong Farmers' Needs and Barriers to Implementing Food Safety Modernization Act Produce Safety Rule (FSMA PSR)

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Introduction: Although previous studies indicated the need for developing hands-on practices for Hmong farmers, the current Food Safety Modernization Act (FSMA) training program does not address this issue.

Purpose: The study was to investigate the specific needs and barriers for hands-on training on food safety practices from the perspectives of Hmong farmers and Produce Safety Alliance (PSA) trainers.

Methods: Six Hmong farmers and six PSA trainers, who had previously worked with Hmong farmers, were recruited for the project. Each farmer was interviewed for 60 minutes to determine their specific needs and barriers in practicing food safety practices. The interviews were facilitated by a translator who is Hmong and speaks fluent English. Each PSA-certified trainer was also interviewed for 60 minutes to discuss their experience and challenges in training Hmong farmers on FSMA PSA. All interviews were audiotaped, transcribed verbatim, and used to preliminarily index concepts and themes.

Results: Washing stations, storage containers, storage rooms, coolers, chemicals (e.g., test strips), and covering structures (e.g., shelters) were identified by the Hmong farmers as items needed to implement food safety practices on their farms. Both farmers and PSA trainers indicated that cost is a significant barrier that prevents Hmong farmers from adopting FSMA PSR practices. Some farmers also mentioned that record-keeping is a challenge, while others found it difficult to understand the FSMA PSR training itself. The PSA trainers identified the lack of standard operating procedures and understanding of food safety practices as major barriers to practicing food safety. Washing stations, storage containers, and coolers were identified as the most beneficial tools for practicing food safety.

Significance: By understanding the needs and barriers for developing hands-on practices for Hmong farmers, this study contributes to further reaching and empowering underserved audiences, who would be able to sell safe food to their customers.

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P2-12 Exploring the Effects of Cultural Values on Food Safety Modernization Act (FSMA) Training: A Comparison between Hmong Farmers and Produce Safety Alliance (PSA) Trainers in the United States

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Introduction: While cultural differences between Hmong and Americans present unique food safety challenges, the current Food Safety Modernization Act (FSMA) training program does not adequately address the needs of Hmong farmers.

Purpose: The study was to (1) explore the influence of Hmong cultural values on FSMA training and related behaviors and (2) compare the variables influencing the behaviors of Hmong farmers regarding FSMA training on their farms from the perspectives of both Hmong farmers and Produce Safety Alliance (PSA) trainers.

Methods: First, six Hmong farmers from southwest Missouri were recruited for a 60-minute interview to investigate the Hmong cultural values that influence food safety practices on their farms. All interviews were facilitated by a translator who is fluent in both Hmong and English. Second, six PSA-certified trainers who had previously worked with Hmong farmers were recruited for a 60-minute interview to share their training experiences with this population. All interviews were audiotaped, transcribed verbatim, and used to index concepts and themes. Lastly, observations of food safety handling behaviors were conducted at five Hmong farms.

Results: Nine Hmong cultural values were identified through individual interviews with 12 Hmong farmers and PSA trainers. Respect, authority, trust, family-oriented, and competitive values were mentioned most frequently by participants. Most participants reported being satisfied with the current FSMA training program, but some Hmong farmers expressed a need for accommodations (e.g., hearing aids) and pointed out difficulties in following FSMA-recommended food safety practices (e.g., cleaning and sanitizing produce). Meanwhile, some PSA trainers expressed concerns with training terminology, length, and format. Based on observations, water supply, domestic animals, and trash were identified as food safety concerns on Hmong farms.

Significance: This study shed light on the influence of traditional cultural values on food safety training in local Hmong farm communities, providing insights from both the perspectives of Hmong farmers and PSA trainers.

P2-13 Using Smart Glasses in Veterinary Inspections in Cows Farm for Animal Welfare and Diseases Prevention and Control: An Italian Pilot Experience in 2022

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Introduction: During Covid-19, health authorities worldwide utilized smart tools to conduct food safety inspections. In EU in April 2021, the new Animal Health Law to manage and prevent the animal welfare and the spreading of transmissible animal diseases became effective. We conducted a pilot using smart glasses to check animal welfare and to conduct farmers' inspections as requested by the EU Reg. 429/2016.

Purpose: Verify the usability and limits of smart glasses remote veterinary inspections in animal farming.

Methods: We select N.3 different types of Cow Farms using smart glasses to verify different activities. One vet on the ground, and one in a remote location: N.1 Intensive Milk Farm with 88 cows, checking the milking room hygiene; N.2 Wild breeding meat cows farm of Tuscan Chianina breed of 200 animals, during blood collection activities, and N.3 Intensive Milk Farm with 80 cows, observing cows' rest area, feeding area and nursery area. Mobile phone and external router Wi-fi were used for internet connection.

Results: A K.P.I. was settled from 0 (insufficient) to 5 (success). Remote Veterinary inspection was: (5) successful in Farm N.1 for quality of photos, video and milking area hygiene checklist execution; (3) good in Farm N.2 for lack of Wi-fi connections in wild area but able to make photos and video during blood collection; (4) very good in Farm N.3, able to conduct welfare, behavior and health checklist requested by the National and EU Law. Many past issues like noise control, Wi-fi connection and camera vision are actually solved in new available smart glasses.

Significance: New Smart Glasses are able to be used in Veterinary Inspections in an easy way and also could became an alternative tool to be used in restricted area access in case of epidemy or pandemy as experienced during covid-19.

P2-14 Knowledge, Attitudes, and Perceptions of Ultraviolet-Light Technologies for Agricultural Surface Water Decontamination by Produce Growers in Kansas and Missouri

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Poster

Introduction: Ultraviolet-C light (UV-C) technology is used extensively for drinking water treatment but has yet to become popularized in the agricultural sector. Particularly in Kansas and Missouri, factors contributing to this lag are largely unknown. Although the fresh produce industry is still developing in these states, growers have expressed interest in non-chemical alternatives for agricultural water decontamination. Thus, it is imperative to understand the major driver(s) for its adoption (or lack thereof).

Purpose: The purpose of this study was to determine which demographic factors most impact the attitudes of fresh produce growers in Kansas and Missouri towards the adoption of UV-C light technology for agricultural water treatment.

Methods: A survey instrument was designed in Qualtrics (KSU IRB Approval #10913). Grower knowledge of UV-C light was measured using five close-ended constructs evaluated on a binary scale (1=correct,0=incorrect). An overall attitude score was calculated from eight constructs using a 5-point Likert scale (Strongly Agree/Strongly Disagree). This score was further separated into perceived ease of use, perceived usefulness, and perceived resource availability. The demographic information collected included: state, age, farming experience (years), education, farm size (acres), farm income (USD), and training certification(s) held.

Results: Eighty-two valid responses were received which is similar to previous studies in the target population. The data indicated a large variation in grower knowledge of UV-C (M=2.61, SD =1.32). Stepwise regression (n = 62) revealed that overall attitudes were most influenced by grower knowledge of UV-C (P < 0.0001), farm size (P = 0.0199), farm income (P = 0.1047), and state (P = 0.1237). Growers perceived cost (33.3%; n=27/81) and technical skills (30.9%; n=25/81) required for UV-C systems as major barriers to its implementation and 34.6% (28/81) felt it wasn't appropriate for their operation.

Significance: The data improves the current understanding of what factors impact the adoption of UV-C technologies for agricultural water decontamination.

P2-15 Validation of a Kombucha Recipe: The Integration of Teaching and Extension

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Introduction: Food microbiology classes are a great setting to involve students in experimental research to learn about the survival of foodborne pathogens in food and their implications on public health. During the food microbiology coursework at the University of Georgia (UGA) and at Kansas State University (KSU), students were given a final project that integrated teaching with a research need previously identified by Extension.

Purpose: To integrate teaching and Extension through a hands-on experience with real-world scenarios for students and validate a kombucha recipe.

Methods: The need for a validated kombucha recipe was identified by both the National Center for Home Food Preservation and Extension. Students participating in a senior food microbiology class at UGA and KSU were exposed to this challenge study and worked in teams to conduct the experiment,

collect, and analyze data. A written report and oral presentation were delivered at the end of the study. To assess learning outcomes, evaluation data were collected utilizing a survey (IRB ID PROJECT00000045).

Results: Sixty-six students participated in this study. Thirty-two (48%) students responded to the survey, of which 62% were undergraduates and 38% were graduate students. A total of 55% of students strongly agreed to have engaged in critical thinking during this project, and 66% prefer to take a class that includes a real-world challenge over one that includes a fictional problem. Seventy-two percent of the students reported the overall experience as good or excellent and appreciated the "Hands-on experience with real-life projects" and exposure "to actively participating in your own learning."

Significance: Employing students in projects driven by real-world research questions not only gives them the chance to acquire important skills needed for employment but also provides value to the community through the knowledge and data generated in class.

P2-16 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=1,336) 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=60) 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 (21.73/25), were significantly higher than pre-test score means (18.28/25), indicating an increase in knowledge after participation in the training (t=-8.69, 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, post-harvest water, and worker training required the most improvements and 46.67% met the requirements, 43.33% needed minor improvements, and 10% 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.

P2-17 Produce Safety in Hydroponic and Aquaponic Operations: Multimedia Educational Resource Development

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Introduction: In 2019, the Northeast Center to Advance Food Safety (NECAFS) identified the leading need among its members regarding produce safety education: produce safety guidance specific to hydroponic and aquaponic (HP/AP) operations.

Purpose: To create produce safety educational resources tailored to HP/AP operations, to then aid in adoption of best practices and compliance with the Food Safety Modernization Act Produce Safety Rule (FSMA PSR).

Methods: We collected relevant existing educational material, then held calls with growers, educators, researchers, and industry to gather an understanding of produce safety knowledge needs and of current educational and research efforts pertaining to hydroponics and aquaponics. This process showed that small- and medium-sized HP/AP operations are, generally, in the very beginning stages of thinking about produce safety with limited produce safety knowledge. We solicited broader stakeholder feedback on this idea and assembled an Advisory Board of subject matter experts. Topic-based factsheets went through several rounds of revision including feedback from the Advisory Board and other stakeholders. Within the subject areas defined by the factsheets, collaborators at Michigan State University (MSU) identified key behaviors that on-farm workers would need to incorporate to ensure a safe product and began developing interactive video content.

Results: NECAFS developed a series of 5 topic-based factsheets that discuss the implementation of best practices and FSMA PSR compliance in the context of HP/AP operations, released in February 2023. MSU supplemented these factsheets with interactive video content to extend and enhance the educational value of the print materials. This work will continue to expand through a USDA NIFA Food Safety Outreach Program grant awarded to NECAFS in 2022.

Significance: As the climate and global economy evolve, hydroponic and aquaponic production of fresh fruits and vegetables will continue to increase. Fresh produce contributes significantly to the burden of foodborne illness, and protecting it begins at the farm.

P2-18 Hands-on Food Safety and Regulatory Training for Members of Shared-Use Commercial Kitchens in Florida

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Introduction: Food entrepreneurs who utilize shared-use commercial kitchens (e.g., incubators, accelerators) often seek food regulatory or food safety guidance as they launch their food businesses. In response, collaborators from the University of Florida and Florida International University created a unique hands-on curriculum that provides a basic overview of state and federal food regulations and outlines food safety plan examples that meet FSMA's Preventive Controls for Human Food Rule standards.

Purpose: This project aims to deliver the training to target audiences and assess short- and medium-term knowledge gains and impacts.

Methods: In 2021 and 2022, a total of 11 training events were held using the new curriculum for 139 participants. The trainings include seven 1-day workshops intended for food business owners/managers working out of shared-use kitchens, three half-day workshops for employees of these food businesses, and one 1-day in-service training targeted towards county Extension Agents within the UF system. At the end of each event, participants completed an evaluation form where they reported their knowledge in six topic areas related to food regulations and food safety before and after the training using a five-point Likert scale. All results were analyzed using a paired t-test.

Results: Data collected from the 1-day workshops (n = 72) indicated a significant increase (P < 0.001) in perceived knowledge of participants across all topic areas with an overall average of 2.69 pre-training and 4.37 post-training. Results from the in-service training (n = 11) and half-day workshops (n = 56) also showed significant increases (P < 0.001) in perceived knowledge for their respective audiences.

Significance: When food entrepreneurs working in shared-use kitchens better understand these topic areas, it helps limit the potential for adverse food safety events occurring in the shared environments and better prepares them for regulations they may face as their businesses grow.

Poster

P2-19 Hybrid Sanitation Programming for Small Processors Guided by Industry Feedback

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❖ ***Developing Scientist Entrant***

Introduction: There is an absence of scale-relevant, hands-on training materials that prepare small processors for compliance with the Preventive Controls for Humans Foods Rule.

Purpose: Identify sanitation-specific training needs of small food processors, by determining gaps in sanitation knowledge, so relatable and relevant hands-on training activities can be designed.

Methods: A survey was distributed to 16 sanitation experts, auditors, inspectors, and sanitation supply companies. The survey included 3 open-ended text response questions and one ranking question. Questions asked the participants about their current job role, commonly observed sanitation practices that they see performed incorrectly or not at all, common misconceptions or questions, and to rank the most top 7 valuable training activities (previously determined based on industry feedback). Survey results were comparatively analyzed to determine top sanitation gaps.

Results: Top observed nonconformances included a lack of knowledge about how to perform cleaning and sanitizing (i.e., how to do it and which chemicals and tools to use) (6 responses), a lack of testing chemical concentrations (6 responses), and a lack of understanding of the difference between cleaning and sanitizing (5 responses). One expert noted that observed deficiencies in sanitation knowledge often led to behaviors that increase the risk of cross-contamination. Common questions from small processors about cleaning and sanitizing included how to use cleaning chemicals, including contact time requirements, the difference between wet and dry cleaning, and cleaning and sanitizing frequency. Results from sanitation experts reported that the top three training topic priorities should include: 1) physical differentiation between cleaning and sanitizing (6 responses), 2) determining the appropriate sanitizer for a specific operation (5 responses), and 3) how to test sanitizer concentrations (5 responses).

Significance: Soliciting input from industry food safety practitioners aims to ensure that the design of in-person training activities is relevant to increasing the efficacy of sanitation programs in small food processing facilities.

P2-20 SALSA to BRCGS START!: Development of a Tool for SME Food Manufacturers to Transition Towards More Complex Food Safety Certification

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Introduction: Research has established the need to assist small and medium-sized (SME) food-manufacturing businesses to select the most appropriate food safety assurance scheme for their business and to support SMEs transition towards more complex certification. In the UK, there are two prominent schemes for SMEs: the SALSA (Safe and Local Supplier Approval) standard is a robust and holistic food safety scheme, appropriate for SMEs; and the BRCGS START! programme is specifically for SMEs where the full BRCGS Food Safety standard may not be practical/required, it is seen as a stepping-stone from SALSA towards the full BRCGS standard.

Purpose: Develop tools to enable SME food manufacturers to transition from the SALSA standard to the BRCGS START! programme.

Methods: The SALSA and BRCGS START! standards were reviewed. Additional requirements when transitioning from SALSA to BRCGS START! were established. Findings informed development of an audit-interview schedule; this was conducted with SALSA certificated SMEs seeking to transition to the BRCGS START! programme (n=6) to establish perceptions regarding complying with the additional clauses. Cumulative findings informed the development of tools to support SMEs with scheme transition.

Results: Sixty-six additional clauses were identified and investigated. Eighteen "clauses of concern" for transitioning businesses were identified. Findings were utilised to develop tools, including: a decision-tree for businesses to select the most appropriate food-safety assurance scheme, a SALSA-to-BRCGS START! transition checklist and self-assessment tool, and a series of webinars by SALSA, BRCGS and the research team to support SMEs with scheme progression and transition.

Significance: SMEs often lack the resource of larger businesses to meet the requirements of complex food safety assurance schemes, this novel study has successfully developed practical tools that are now utilised by BRCGS and SALSA scheme owners to help SMEs identify the most suitable assurance scheme and navigate through these schemes as they evolve.

P2-21 Exploring the Mediating Role of Food Safety Culture in Achieving Hand Hygiene Compliance to Inform Bespoke Food Manufacturing Interventions

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❖ ***Developing Scientist Entrant***

Introduction: Frequently implicated in foodborne illness outbreaks, handwashing is a critical component in supporting food safety. However, ubiquitous organisational cultures may mediate behaviour to a greater or lesser extent than is realised or anticipated.

Purpose: Assess hand hygiene (HH) practices and food safety culture in food manufacturing and processing (high-risk/care and low-risk departments) determining the mediating role of culture on behavioural compliance to inform development and implementation of bespoke interventions.

Methods: Underpinned by the GFSI food safety culture framework, a longitudinal pre/post test triangulated mixed-methods study design combined findings from in-depth interviews (n=23), surveys (n=193), focus groups (n=4), observations (150h), document review (n=112) and microbiological hygiene indicator assessment (n=2685) to facilitate bespoke HH intervention implementation (n=6) and outcome evaluation.

Results: In-depth interviews depicted positive attitudes towards food-safety (i.e. aspects of the vision and mission dimension), however, priorities were focused on productivity. Consequently, focus groups revealed that HH expectations were unclear (i.e. consistency), complicated by a company-wide reliance on glove-use during production and ambiguous instructions regarding handwashing as identified in company procedures/signage (i.e. people dimension). Surveys suggested that food operatives had knowledge of hand hygiene requirements but rarely applied the same in practice (i.e. hazard and risk awareness). Post-intervention findings indicated that bespoke co-created interventions, pre-production entry increased handwashing proficiency and durations (from an average 9s to in excess of 15s) (i.e. adaptability).

Significance: The mediating role of food safety culture on HH manifested inconsistently which, without a triangulated approach to assessment, would otherwise have gone unnoticed. A mixed-method technique enabled broader data capture representative of the prevailing food safety culture and consequent behavioural outcomes. Hand hygiene compliance in food manufacturing is therefore influenced by food safety culture, typically mirroring the dimensional characteristics portrayed by management, necessitating bespoke collaborative interventions to improve HH standards.

P2-22 Identifying Food Science and Technology Shortages in Food and Drink Manufacturing and Processing Businesses in Wales, UK

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Introduction: The food and drink manufacturing, and processing (FDMP) industry is one of the largest manufacturing industries, and it employs millions of people worldwide. For FDMP to be sustainable, competitive, and profitable, food science and technology (FST) skills are vital in areas such as food safety, legislation, and innovation. Recent data has indicated shortages of people with FST skills in many countries such as Canada, New Zealand, and the UK.

Purpose: This study aims to identify the FST labour challenges currently facing the FDMP industry in Wales, UK.

Methods: Electronic self-completed questionnaires were distributed to all FDMP owners and managers in microbusinesses, Small to Medium Enterprises (SMEs) and large businesses in Wales, identified using the Food Innovation Wales' database. The questionnaires focussed on FST skills needs and gaps and on FST recruitment needs (n=50). A follow up, in-depth telephone interview, collating qualitative data on FST skills needs (n=12) was also conducted.

Results: Cumulative findings showed that of the (n=50) FDMP businesses, 52% highlighted skills shortages in areas such as technical, food safety management, labelling, food quality, and legislation. Businesses identified 14 different FST skills gap areas including factory design and fabrication, sensory analysis, and allergens. The in-depth telephone interviews (n=12) also confirmed shortages in technical areas. Quote: "we are weak in that area, we have one person, he does all of the technical calculations and recipe management, if he was away, we haven't transferred the knowledge to others" Company G.

Significance: This study highlights FST labour challenges in respondent businesses. There are recruitment issues and skills shortages in FST, the area responsible for innovation, food safety and legislation. This information can be used to inform industry support centres and government to enable better training, recruitment, and policies.

P2-23 Enhancing Food Safety Outreach to Underrepresented Communities through the Development of Targeted Training Material

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Introduction: There have been over 2,000 Produce Safety Alliance (PSA) Grower Trainings in the United States. This has resulted in over 40,000 growers being trained. However, underserved minority groups are often overlooked. In addition, the prescribed training leaves little time for hands-on activities that would reinforce understanding of the concepts addressed. In this project, growers identified hands-on activities and visual aids (flyers or posters) as their preferred educational approaches.

Purpose: The project's overall objective was to develop interactive food safety educational materials for underserved communities. These interactive activities expand topics of food safety education concerns identified for the target audience.

Methods: Underserved growers in Alabama indicated that they would appreciate food safety training in a hybrid version of the PSA Grower Training Course. Specifically, they identified the type of training as a lecture and the other part as an on-farm demonstration or walkthrough of the Produce Safety Rule. As a result, the food safety topics of concern identified were: (1) Wildlife, Domesticated Animals and Land Use, (2) Agricultural Water, and (3) Worker Health, Hygiene, and Training.

Results: Program assistants developed interactive activities and facilitator guides for each topic. Other supplemental resources were also designed, such as videos demonstrating microbiological techniques such as streaking, plating, and environmental sampling. Furthermore, short recorded 5-minute learning modules cover the topics areas of: (1) Produce Safety, (2) Worker, Heath, Hygiene and Training, and (3) Soil Amendments. An Advisory Board of food safety experts reviewed all resources.

Significance: The developed educational material will be used in workshops aimed at meeting the needs of underserved minority groups that are often hard to reach. These individuals will be provided an opportunity to participate in interactive activities that could be used to implement change in their operations.

P2-24 Maize Handling Practices in Guatemalan Communities with High Maize-Based Food Consumption

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Introduction: Rural communities in Guatemala are exposed to mycotoxins, which are secondary metabolites produced by fungi, through high consumption of maize-based foods; improved maize-handling practices can decrease this food safety risk.

Purpose: To identify associations between maize-handling practices, socio-demographic characteristics, and knowledge about mycotoxins.

Methods: A cross-sectional survey of individuals (n=56) living in three rural municipalities in Alta Verapaz, Guatemala, was conducted from February to March 2020; the study terminated early due to the COVID-19 pandemic. A questionnaire to assess socio-demographic characteristics, maize handling practices, and dietary history was administered to mothers after giving birth in local hospitals via interviews by trained health professionals in Spanish, ensuring completeness and accuracy. If mothers could not answer the survey, their husbands were interviewed. Data were analyzed using descriptive statistics and logistic regression; marginal associations were included.

Results: Most participants (75.00%) reported cultivating maize, and more than half of all participants (58.92%) purchased maize at the local market. Overall, 67.86% of participants classified their maize by size (71.05%), color (44.74%), visible damage (23.68%), and/or presence of fungi (5.26%). Women were 0.22 times less likely (95% CI: 0.05 to 1.02; P=0.0536) to classify their maize by visible damage and presence of fungi as compared to men. In addition, few (12.50%) knew fungi could cause maize damage or crop loss in their community. Those who knew fungi can cause damage/loss were 3.84 times more likely (95% CI: 0.72 to 20.58; P=0.1157) to consume damaged maize than those unaware of this issue. Overall, 19.64% of the individuals reported consuming maize with visible damage.

Significance: While small sample sizes limit interpretation of association, these findings suggest tailored food safety interventions are needed to improve maize-handling practices in rural communities in Guatemala. Mitigating the risk of mycotoxin exposure will directly influence the food security of those communities.

P2-25 Food Safety Mistakes When Preparing Breakfast: Findings from an Observation Study

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Introduction: Observing consumers prepare meals in a test kitchen environment can help to identify areas where improvements are needed in consumers' food safety practices.

Poster

Purpose: To quantify consumers' adherence to food safety practices when preparing raw breakfast sausage, shell eggs, and cantaloupe in a test kitchen environment.

Methods: Participants (n = 125) were observed while being video-recorded during meal preparation. Participants' behaviors were coded to measure adherence to food safety practices. In post-observation interviews, participants were asked about their usual food preparation practices when cooking at home.

Results: Fifty percent of participants were observed using a thermometer to check doneness of the sausage patties. During the interviews, some participants reported this is not their usual behavior when cooking at home. On average, participants used a thermometer to check the doneness of 3 of the 4 sausage patties and most failed to insert it in the proper location. Similar to other observation studies, handwashing compliance was low. The rate of successful handwashing attempts was 44% before meal preparation and 17% during meal preparation. The most common reason observed for not successfully washing hands was failing to rub hands with soap for at least 20 seconds. Less than half (43%) of participants attempted to wash their hands after cracking eggs; of those, only 1% successfully did so. Among participants making scrambled eggs, 69% reported cooking them until the yolk was firm; this rate decreased to 20% among participants who cooked fried eggs. The rate of observed washing attempts for the cantaloupe rind was low (25%).

Significance: Many participants made food safety mistakes when preparing breakfast. While some participants were aware of recommended practices, they did not always properly execute the practices when cooking, suggesting the need for additional messaging explaining the "how" and the "why" to motivate behavior change.

P2-26 What We Know about Consumers' Use and Understanding of Manufacturer Cooking Instructions on Meat and Poultry Products: Findings from Focus Groups

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Introduction: Many manufacturers of meat and poultry products provide voluntary Manufacturer Cooking Instructions (MCI) to provide guidance on how to properly prepare their products. Limited information is available on how consumers use these instructions.

Purpose: To learn how consumers understand and use MCI when preparing raw and processed meat and poultry products.

Methods: We conducted eight virtual focus groups with U.S. English-speaking adults (segmented by education and age) who have experience preparing frozen, processed meat or poultry products.

Results: When cooking raw meat and poultry products, many participants do not rely on cooking instructions because they already know how to cook the product or rely on recipes instead. When preparing processed meat and poultry products (ready to eat or not ready to eat [NRTE]), almost all participants typically follow the cooking instructions the first time they prepare the product to ensure they cook it correctly, but do not always refer back to the instructions when preparing it again and rely on experience. Participants noted differences between the MCI and the mandatory safe handling instructions label (SHI)—saying the MCI provides information for preparing or cooking the product whereas the SHI provides information on food safety practices. Additionally, many participants correctly understood that microwaving is not recommended for NRTE products because they are uncooked and would not cook properly. Many participants considered the MCI to be more useful than the SHI, however they said having both the MCI and SHI is helpful for inexperienced food preparers.

Significance: The focus group findings suggest that it would be useful for MCI to include information on food safety practices, such as how to use a thermometer and proper internal temperatures, since consumers refer to this information the first time they cook a processed meat/poultry product.

P2-27 Hand Wash Practices after Handling Breakfast Sausage and Eggs during Meal Preparation

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Introduction: Inadequate hand washing can be a contributing factor to foodborne illnesses in the home kitchen. After handling raw meats, poultry, and eggs, hands can spread potential foodborne pathogens during meal preparation. However, the frequency of handwashing events after handling different food items during meal preparation is not well studied.

Purpose: The purpose of this study is to compare hand washing frequency and compliance after the cracking of raw eggs and the handling of raw breakfast sausage during meal preparation.

Methods: Participants (n=125) were asked to prepare a simple breakfast meal containing eggs and breakfast sausage in a consumer-style kitchen while being observed and videotaped by researchers. Researchers then coded and analyzed the videos for handwashing frequency and compliance according to CDC guidelines.

Results: Out of the 192 required hand washing events after cracking eggs, participants attempted hand washing 44% of the time. Of these attempts, 67% used soap and 33% rinsed only. Out of the 150 required handwashing events after handling raw breakfast sausage, participants attempted handwashing 46% of the time and of these attempts, 90% used soap and 10% rinsed only. The difference in soap use is statistically significant ($p<0.05$).

Significance: Although this study demonstrates similar hand washing attempts after handling of raw sausage and raw eggs, participants were more likely to use soap after handling raw sausage compared to raw eggs. These results suggest more research is needed into consumer handling of specific food items and consumer perceptions in order to increase the effectiveness of food safety messages.

P2-28 Engaging the Young, Old, Immunocompromised, and Pregnant People on Food Safety Matters

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Introduction: Young, old, pregnant, and immunocompromised (YOPI) people are some of the most vulnerable communities to foodborne illnesses. New Zealand Food Safety (NZFS), a business unit within Ministry for Primary Industries has committed to improving its understanding of consumer information needs and behaviour so that foodborne illnesses in New Zealand can be reduced.

Purpose: To develop a better understanding of the knowledge, understanding and uptake of food safety information among New Zealand's vulnerable communities and to inform the development of communication and engagement approaches about food safety to these groups.

Methods: Twenty focus groups were recruited to assess food safety understanding of young, old, immunocompromised, and pregnant people as well as health practitioners. The research questions were guided by a rapid review of relevant literature.

Results: Findings from this research to date has revealed gaps in understanding and measurement of food safety matters. The risks of foodborne illness were generally underestimated with perceptions being critical to understanding YOPI consumers behaviour and food safety practices. Recruitment of focus groups proved to be more challenging than expected which, in part, may have been due to the COVID-19 pandemic.

Significance: Insights into YOPI knowledge, understanding and uptake of food safety information will help inform the development of appropriate and effective communication and engagement approaches on food safety matters to YOPI consumers.

Poster

P2-29 Assessment of Food Safety Education Resources Available to School-Aged Children in Canada

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Introduction: The Government of Canada is increasing outreach and education efforts aimed at consumers to help address food safety knowledge and behaviour gaps. One new project underway involves the development of food safety educational resources for school-aged children in Canada.

Purpose: To assess food safety educational resources available to primary and secondary school teachers in Canada to inform the development of quality resources that will be fit for classroom use.

Methods: Data gathered from a gap assessment, curriculum research and educator consultation sessions were triangulated to assess the food safety resources currently available.

Results: The assessment revealed a pressing need to create food safety resources for primary school teachers and students. Given the wide scope of resources needed, the recommendation is to focus at first on creating resources for grades 4–6. The research indicated that this is when students begin to learn about topics related to food safety (e.g., microorganisms, pathogens, personal responsibility for safety and maintaining a healthy body). This age group is also more interested in learning about food safety as they become more active in food preparation in the home. Feedback received from focus group sessions highlighted the need for resources such as games, graphics, videos and hands-on activities. Educational resources will need to be inclusive for all learners and should use engaging visuals, sequencing and repetition. They will also need to consider the cultural diversity and the social-emotional impact the information may have on students.

Significance: Classroom-ready education resources are needed to teach safe food practices to school-aged children in Canada. Involvement of students and teachers will be required in the development of these resources to ensure that they meet the learning outcomes of Canadian provinces and territories and are relevant to existing curricula.

P2-30 Thinking Outside of the Box: Food Safety and Nutritional Information in UK and U.S. Meal-Kit Recipe Boxes

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Introduction: Growing in popularity, meal-kits give consumers the opportunity to prepare home cooked meals by providing boxes of fresh, measured ingredients with step-by-step recipe cards. Currently, little is known about the food safety and nutritional information included in meal kit recipes.

Purpose: Determine the provision of food-safety and nutritional information in UK and US meal kit recipes.

Methods: Cumulatively, 485 meal-kit recipe cards (UK=359; US=126) from 19 providers (UK=8; US=11) were obtained from consumers via social media. A Qualtrics tool was developed to review inclusion of food-safety and nutritional information.

Results: Although all eight UK and 10 of the 11 US meal kit providers included some form of food-safety related information, the information was often inadequate to ensure domestic food-safety. For example, of meal kit boxes that included perishable ingredients; 51% of UK recipes specified the need for chilled storage, whereas only 12% of US recipes provided such information. Only one recipe (from UK) referred to recommended temperatures (≤5°C). Advice on washing fruit and vegetables were positive, with 88% of UK and 82% of US recipes referring to the practice. Handwashing before starting food preparation was stated in 46% of UK recipes but none of the US recipes. UK recipes provided handwashing prompts after 47% of occasions requiring handwashing such as after preparing raw poultry, while in US handwashing was only referenced on 6% of recipes. Subjective guidance for cooking adequacy were found; with UK statements (*n*=1306) focusing on visual assessment of colour (35%) while US statements (*n*=236) referred to cooking duration (40%). Nutritional information was provided by six UK providers and seven US meal-kit providers.

Significance: This study has identified differences in food-safety information communication by US and UK meal kit providers. Although food-safety information was more frequently included in UK recipes, there is a need to understand if, and how, consumers engage with such information when following meal kit recipes.

P2-31 "I Express Breastmilk. What's Your Superpower?": Hygiene Perceptions and Practices of UK Mothers Expressing Breastmilk for Infants

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Introduction*:* For optimal development, health, and maternal wellbeing, it is recommended that infants should receive only breastmilk for the first six months. Multiple reasons exist why some mothers can't breastfeed directly and choose to express; however, when expressing breastmilk for an infant, parents have additional hygienic responsibility for safely expressing and storing breastmilk.

Purpose*:* To explore mothers' hygiene perceptions and practices when expressing, storing and providing breastmilk for infants and establish trusted sources of information.

Methods*:* A semi-structured interview schedule was developed using the Behaviour Change Wheel COM-B model, and incorporated principals of the British Dietetic Association guidelines for the collection, storage, and handling of maternal expressed breastmilk. Online/telephone in-depth interviews were undertaken with mothers (*n*=40) who had exclusively and non-exclusively expressed breastmilk for an infant.

Results*:* Reasons for expressing breastmilk included premature-birth, hospitalisation, increasing milk supply, involving others in feeding, returning to work, and donating to milk banks. Confusion existed regarding appropriate storage durations for breastmilk, many believed storage duration guidance from UK National Health Service was "too long"; therefore, reported shorter refrigerated storage, or freezing for longer durations. Challenging situations when expressing at work/university were discussed, including fears regarding temperature of workplace refrigerators. Mothers reported obtaining hygiene/storage advice and trusting information from peers on social-media platforms; particularly as healthcare professions were not readily accessible, and questions would be answered immediately on social-media.

Significance*:* Despite research focusing on hygienic practices with powdered infant formula, there is a particular lack of research relating to expressed breastmilk. This study has explored capability, opportunity, motivation, and behaviour in relation to expressing breastmilk and has determined potentially unsafe storage temperatures in communal workplace refrigerators.

P2-32 Exploring the Feasibility of Using a Simulated Environment to Enhance Food Safety Training and Research Opportunities

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Introduction: In the food manufacturing sector, real-life scenarios can't always be accessed to deliver training, as production is the priority. However, immersive user-testing facilities such as the Perceptual Experience Laboratory (PEL) allow real-life scenarios to be replicated using a 200° projection environment to stage seamless field-of-view media around the user, whilst controlling multi-sensory contextual variables under laboratory conditions. Consequently, there is a need to explore the feasibility of using the PEL for food safety training.

Purpose: Explore the feasibility of using a simulated environment to deliver food safety training within food industry conditions that are not readily accessible.

Poster

Methods: The research was conducted across two matched conditions: 1) the Food Industry Centre (FIC) bakery, and 2) the PEL, which replicated the FIC bakery environment. In both conditions, food industry experts (*n*=16) wearing eye-tracking glasses were presented with an identical series of food safety hazards and were required to verbally identify them. Post-participation perceptions of the PEL were obtained, with reference to future applications.

Results: No significant difference (p>0.05) was found between the real bakery and the PEL bakery when completing the verbal food safety hazard reporting task. The comparable results between the PEL and the real bakery evidence the reliability of the PEL as a tool for delivery of future training activities. Additionally, analysis of eye-tracking data was able to pinpoint knowledge gaps if critical elements were observed but not reported by participants. The study also demonstrated the stability of the PEL over the commercial bakery as an environment that could be controlled more effectively, improving training consistency. Participating food industry experts discussed how the PEL could be utilised to present food manufacturing *"audit training scenarios"* to *"improve auditor consistency"*.

Significance: The study demonstrates how novel technologies such as simulated environments could be utilised to train individuals to implement food manufacturing internal audits more effectively.

P2-33 Teaching Food Product Development: The Importance of Food Safety and Quality Assurance Incorporated into Undergraduate Curriculum

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Introduction: Undergraduate food science students interested in becoming food product developers and some faculty new to food product development (FPD) teaching with their lack of food safety knowledge make them prime candidates for food safety education modules, specifically for FPD. Two recent surveys (McGraw-Hill and Forbes) identified clear gaps between the expectations of food industry employers and food industry stakeholders related to new food science graduates' FPD skills and performance.

Purpose: The present study aimed to investigate perspectives of faculty and industry professionals on content that should be taught to have competent food product developers.

Methods: Data were collected via a remote survey. The survey was completed by 53 faculty and 69 industry professionals (n=122) across the United States. Survey completion was voluntary and anonymous. The inclusion criteria included being a faculty in a higher education institution or a professional working in the food industry (past or present).

Results: There are competency gaps in the education of both FPD higher education faculty and graduates going into the food industry as food product developers. Several industry professionals believed that there is a significant disconnect between academia and industry in preparing students for FPD careers (32/69 respondents). According to industry professionals, the most important skills needed are food chemistry (41/69 respondents), food safety (35/69 respondents), food microbiology (30/69 respondents), food processing and sensory (27/69 respondents), and quality control/assurance (26/69 respondents), in addition to soft skills and a general understanding of the food business.

Significance: The results indicated that the FPD faculty must incorporate food safety and quality assurance into FPD curriculum at the undergraduate level. Food science graduates need a basic level of understanding of food safety and quality assurance to successfully develop food products. Therefore, this study highlights the need for a strong food safety curriculum to be developed to be used specifically in FPD courses.

P2-34 U.S. Consumers' Perceptions, Behaviors, and Attitudes Toward Tree Nut Food Safety across Demographic Groups

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❖ ***Developing Scientist Entrant***

Introduction: Risk perception, knowledge, and attitudes can drive consumers' food handling and behavioral change. Multiple elements may influence consumers' perception of food safety, including age, gender, level of education, and diet.

Purpose: To identify (1) consumer demographic characteristics of perceptions of tree nut food safety, and (2) handling behaviors related to the use of raw (not roasted, blanched, or pasteurized) tree nuts, especially in the preparation of nut-based dairy analogs (NBDA).

Methods: In January 2022, researchers developed an online survey and distributed it to consumers (n=981) who engaged in soaking raw tree nuts or making NBDA in the home. The survey questions focused on consumers' handling, perceptions, and knowledge of tree nut preparation, as well as their preferred sources of food safety information. Researchers then conducted a quantitative analysis to identify handling practices and perceptions within different demographic groups.

Results: Most participants were female (63%), between ages 25 and 44 (59%), held a bachelor's or more advanced degree (43%), and followed no specific diet (52%). The analysis found statistical significance that male participants (32%) were more likely than females (27%) to use recalled tree nuts that they had purchased. Participants between the ages of 35 and 54 (23%) were significantly more likely than other age groups to perceive a higher level of food safety risk from raw tree nuts. Participants who held bachelor's or more advanced degrees on average scored higher on tree nut food safety knowledge questions than those without such degrees (OR=1.07; $P<0.05$). Compared to those who followed a specific diet (e.g., vegetarian, vegan, keto) (n=470), participants who did not follow a specific diet more often used raw tree nuts to make NBDA but were less concerned about loss of health benefits during pasteurization (OR=2.08; $P<0.05$).

Significance: The data will support the development of more customized educational materials on soaked nuts and NBDA.

P2-35 Prevalence and Antibiotic Resistance of *Escherichia coli* Pathotypes Isolated from Fecal Samples of Cattle in Central and Northeastern Mexico

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✦ ***Undergraduate Student Award Entrant***

Introduction: Antibiotic resistance in pathogenic bacteria has increased in part due to the overuse of antibiotics as prophylactics in the livestock industry.

Purpose: To determine the prevalence and antibiotic resistance of *E. coli* strains, isolated from feces of beef (BC) or dairy (DC) cattle, from two states of Aguascalientes (AG) and Nuevo Leon (NL) in Mexico.

Methods: One hundred and ten bovine stool samples were collected (BC-AG n=30; DC-AG n=20; BC-NL n=30; DC-NL n=30). *E. coli* was isolated by dissolving 1g of feces in PBS, and streaked into selective media with subsequent confirmation of colonies on chromogenic media. Multiplex PCR was used to identify diarrheagenic *E. coli* and the Kirby-Bauer technique allowed us to determine the antimicrobial susceptibility of those pathotypes.

Poster

Results: From 550 presumptive colonies isolated, 327 were confirmed as *E. coli*. From these, 11.3% (37/327) were identified as diarrheagenic pathotypes. The most prevalent *E. coli* pathotypes were enterohemorrhagic (24/37, *stx1/stx2/eae*), followed by enteropathogenic (7/37, *lt/stII*), enterotoxigenic (5/37, *bfp/eae*), and enteroinvasive (1/37 *ipaH/virF*). Most pathotype isolates were detected from the samples of cattle raised in AG (28/37). According to the productive purpose of the animals, 21/37 (56.7%) pathotypes were isolated from beef cattle; meanwhile, 16/37 (43.2%) were obtained from dairy cattle. Multi-resistance to penicillin, erythromycin, trimethoprim/sulfamethoxazole, and tetracyclines was observed in 100% of *E. coli* pathotypes. Resistance to colistin was present in 10.8 % (4/37) of isolates from NL.

Significance: We demonstrated that cattle from the study regions are reservoirs of diarrheagenic *E. coli* and showed multi-resistance to several antibiotics. The existence of these pathogenic and multi-resistant pathogens in the food chain is a risk to public health, and actions are needed to reduce their presence and prevent these isolates from causing human and animal diseases.

P2-36 Assessment of the Microbiological Safety of Akkawi Cheese and the Antimicrobial Resistance Profiles of Associated *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: Cheese is a staple food in many low and middle-income countries; however, contaminated cheese can pose a significant public health concern in these countries. Lebanon, a developing country, has been facing well-documented challenges with environmental pollution, food safety, and the dissemination of antibiotic resistance (ABR).

Purpose: We assessed the microbiological safety and the occurrence of antibiotic-resistant *E. coli* in Akkawi, a white brined cheese that is widely consumed in Lebanon.

Methods: A total of 50 Akkawi cheese samples were collected from 16 major retail stores across Beirut, the capital of Lebanon. Samples were homogenized (25 g of cheese + 225 mL of buffered peptone water), and 100 µL was plated on chromogenic Tryptone Bile X-Glucuronic (TBX) and Baird-Parker (BPA) agar for the isolation and enumeration of fecal indicators (*E. coli* and fecal coliforms) and *Staphylococcus aureus*, respectively. Bacterial densities (CFU/g) were compared to LIBNOR (Lebanese standards) standards. The disk diffusion assay was conducted using eighteen important antibiotics to determine the ABR profiles of the isolated *E. coli* (n=135). Hierarchal cluster analysis was performed using Morpheus software.

Results: E. coli, fecal coliforms, *S. aureus,* and other staphylococci were detected in 80% (n=40), 86% (n=43), 32% (n=16), and 98% (n=49%) of the cheese samples, respectively. Notably, 80% (n=40) and 32% (n=16) of the samples exceeded the permissible limit of *E. coli* and *S. aureus,* respectively. *E. coli* showed resistance to ampicillin (67% of isolates), amoxicillin-clavulanate (51%), cefepime (21%), cefotaxime (43%), cephalexin (64%), cefixime (18%), doripenem (36%), imipenem (19%), meropenem (34%), gentamicin (40%), kanamycin (36%), streptomycin (58%), tetracycline (41%), ciprofloxacin (5%), norfloxacin (4%), trimethoprim-sulfamethoxazole (39%), and chloramphenicol (29%). Notably, 73% of the isolates were classified as multi-drug resistant (MDR).

Significance: The contamination levels and the circulation of MDR *E. coli* call for urgent action to enhance the safety of the Akkawi cheese.

P2-37 Characterization of *Listeria monocytogenes* Strains Isolated from Costa Rican Fresh Cheese

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Introduction: *Listeria monocytogenes* is present in Costa Rican fresh cheeses and the production environment.

Purpose: To determine invasion capacity and resistance to disinfectants for *L. monocytogenes* strains isolated from fresh cheese in Costa Rica.

Methods: Invasion capacity: Confluent 6 well micro-plates of HeLa cells were inoculated with a suspension of each of the strains. The inoculated cells were incubated for 30 minutes, then washed and treated with gentamicin. The cells were then washed with phosphate buffer and lysed with cold distilled water and vigorous shaking. *Listeria* cells were then enumerated in Tryptic Soy Agar (TSA). The invasion percentage was determined by dividing the CFU/mL of the lysed cell product by CFU/mL of the suspension used for the inoculum.

Resistance to disinfectants: The strains were challenged with different concentrations of three groups of disinfectants: chlorine, quaternary ammonium and peracetic acid. Each bacterial suspension was exposed to the disinfectants and were then incubated in tryptic soy broth (TSB) for 24 hours. After the incubation time, optical density was determined at 620 nm.

Results: All of the analyzed *L. monocytogenes* strains were able to invade HeLa cells. Recovery rates ranged from 35% to 54%. Interestingly, two of the five cheese isolates presented significantly higher ($p<0.05$) recovery rates than a clinical isolate.

Significance: All of the isolates obtained from fresh cheese were able to invade human epithelial cells, which is known to be important in the virulence of *Listeria monocytogenes*. Under laboratory conditions, no resistance to common disinfectants was demonstrated, this can vary in the production environment with the presence of organic matter or an inappropriate use of the products.

P2-38 Prevalence of *Listeria monocytogenes*, *Salmonella* spp., Shiga Toxin-Producing *Escherichia coli*, and *Campylobacter* spp. in Raw Milk in the United States between 2000 and 2019: A Systematic Review and Meta-Analysis

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Introduction: Raw (unpasteurized) milk is available for sale and direct human consumption within some states in the United States (US). Raw milk for direct human consumption cannot be sold or distributed in interstate commerce. Raw milk may contain pathogenic microorganisms that, when consumed without pasteurization, may cause illness, and sometimes may result in death. No comprehensive review for prevalence of bacterial pathogens in raw milk in the US exists.

Purpose: The objective of the present research was to systematically review the scientific literature published from 2000 to 2019 to estimate the prevalence and levels of *Listeria monocytogenes*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC), and *Campylobacter* spp. in raw milk in the US.

Methods: Peer-reviewed studies were retrieved systematically from PubMed®, Embase®, and Web of Science™. Twenty unique studies were selected using the Health Assessment Work Collaborative (HAWC) and included in the systematic review and meta-analysis. Comprehensive Meta-Analysis (CMA, Biostat, Englewood, NJ) was used for random effects statistical analyses and to generate Forest plots to synthesize and visualize raw bulk tank milk (BTM) and milk filters (MF) data.

Results: The average prevalence (event rate) of *L. monocytogenes*, *Salmonella* spp., STEC, and *Campylobacter* spp. in raw BTM in the US was estimated at 4.3% (95% confidence intervals [CIs], 2.8-6.5%), 3.6% (95% CIs, 2.0-6.2%), 4.3% (95% CIs, 2.4-7.4%), and 6.0% (95% CIs, 3.2-10.9%), respectively. The average prevalence of these pathogens in MF was estimated at 13.1% (95% CIs, 3.7-37.6%), 16.0 (95% CIs, 9.9-24.9%), 49.9 (95% CIs, 35.4-64.3%), and 17.5 (95% CIs, 7.7-35.1%), respectively.

Significance: This study provides a transparent and robust summary of the major bacterial pathogens prevalence of raw milk in BTM and MF in the US between 2000 and 2019. This information could be used to better characterize the potential risk of illness arising from the consumption of raw milk in the US.

Poster

P2-39 Effect of Low Iodine Dose in *Staphylococcus aureus* Biofilm Density

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Introduction: *Staphylococcus aureus* is a zoonotic foodborne pathogen frequently found in dairy farm environments. Iodine is commonly used as a teat dip to control this organism during the milking process. Using the correct dose of iodine is critical to prevent microbial dissemination. Not controlling *S. aureus* will render in mastitis dissemination and establishment of the organism on dairy farm equipment and the environment due to biofilm formation.

Purpose: To evaluate lower iodine concentrations used as teat dip to inhibit biofilm formation by *S. aureus*.

Methods: Five *S. aureus* isolates were tested in triplicate. Strains were previously grown in brain heart infusion broth. A 100µl-aliquote of each strain was placed individually into a well of a microtiter plate, treated separately with 0.25% and 0.375% of iodine, and allowed biofilm production by incubating at 37°C for 48h. After incubation, distilled water was used to rinse the wells, and the remaining biofilms were stained with crystal violet. Ethanol was used to solubilize the crystal violet. Optical density (OD) at 580nm was tested to measure biofilm formation/density.

Results: Both iodine concentrations had an effect (P<0.05) in reducing *S. aureus* biofilm formation. Comparing the OD of the biofilms treated with iodine and the average OD of biofilms in the absence of treatment (0.529±0.02nm), 80% reduction in the biofilm density was estimated. Biofilm reduction was not dose-dependent (P>0.05). The average density of *S. aureus* biofilm was 0.092±0.007nm and 0.099±0.01nm when the strains were treated with 0.25% and 0.375%, respectively.

Significance: Concentrations between 0.5% to 1% of iodine are commonly used as a teat dip in some dairies around West Texas. Testing lower concentrations of iodine help to demonstrate the effectiveness of the treatment for microbial control. The iodine dose tested may reduce *S. aureus* colonization on the exterior of the udders, equipment, or environment.

P2-40 Time and Temperature Abuse of Milk in Conditions Representing a School Cafeteria Share Table Does Not Meaningfully Reduce Microbial Quality

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Introduction: Share Tables (STs) are locations in school cafeterias that allow students to return unopened food items, presenting an opportunity to improve food security and reduce food waste. However, concerns have been raised over sharing milk due to possibly accelerating the growth of spoilage microorganisms.

Purpose: To examine whether the microbial quality of milk cartons declines meaningfully during repeated circulation in a representative ST.

Methods: Empirical time data from school bell schedules was used to create a representative worst-case ST: ambient temperature for 125 minutes, repeated over 5 days (d_1-d_5). The initial microbial load was determined by enumerating milk cartons. Eight cartons below the limit of detection [LOD = 2.31 log(CFU/ml)] were selected. Four were inoculated with 2-3 log(CFU/ml) of *Pseudomonas poae*, a gram-negative, psychrotrophic, spoilage organism, and four were not inoculated. All cartons were refrigerated (4.2°C±0.9°C) 24 hours before d_1. Two controls remained in refrigeration, while six experimental replicates entered the worst-case ST (20.3°C±1.2°C, d_1-d_5). Each day, experimental replicates were sampled at the start (*t* = 0 minutes) and end (*t* = 125 minutes) of the ST. Samples were enumerated on Standard Methods Agar and Crystal Violet Tetrazolium Agar. The change in growth was calculated (i) within the ST each day (start of ST_{dn} to end of ST_{dn}), and (ii) during overnight refrigeration (end of ST_{dn} to start of ST_{dn+1}).

Results: All uninoculated milk cartons had no enumerable growth above the LOD of 2.31 log(CFU/ml). Regardless of sharing status, inoculated milk had a significantly ($P < 0.001$) lower average change in growth [0.130±0.081 log(CFU/ml)] during the 125-minute ambient ST condition than during overnight refrigeration [1.0±0.058 log(CFU/ml)].

Significance: Milk's microbial quality is not meaningfully impacted by repeated circulation in a worst-case ST, particularly compared to the effects of overnight refrigeration. Therefore, sharing milk is unlikely to increase spoilage relative to routine school lunch practices.

P2-41 Heat Transfer Model for Milk Temperature for Predicting Quality of Milk Shared in Different School Lunch Service and Storage Conditions

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Introduction: Share Tables (ST) are locations in schools' cafeterias where students can share unwanted food items with other students to reduce food waste and food insecurity. Milk is considered a Time/Temperature Control for Safety food item. Food inspectors and stakeholders have shared concerns about implementing ST in schools as milk may go through temperature abuse and increase the likelihood of spoilage.

Purpose: To evaluate the time-temperature profile of milk under different ST conditions to develop a heat transfer model that predicts the milk temperature under different lunch service lengths and ambient temperatures.

Methods: Five practical storage conditions were tested for serving and storing milk during a typical school lunch service while using ST: ambient temperature (AT), refrigerated metal tray (RT), tray with ice packs (TIP), tray with ice cubes (TIC), and a cooler with ice (CI). Data from the School Nutrition Association and 357 Illinois school bell schedules supported testing three lunch period scenarios long (50 minutes), medium (30 minutes), and short (20 minutes). Two milk carton replicates were stored under each condition using thermocouples to obtain the empirical temperature data. Data were used to determine the heat transfer coefficients (h) and by lumped-capacity analysis then predict the milk temperature.

Results: Five heat transfer models were developed to predict the milk temperature under different storage conditions. The heat transfer coefficients were 2.96 (AT), 2.10 (RT), 1.72 (TIP), 2.73 (TIC), and 0.45 (CI) all in kW/. By applying the lumped-capacity analysis and the exponential decay formula, the models had a root mean square error of 0.50°C (AT), 0.32 (RT), 0.20 (TIP), 0.30 (TIC), and 0.32(CI), for the fitted data.

Significance: Heat transfer equations from this project can be used to predict time-temperature profiles for new storage times and temperatures using the same conditions. Future work can use these predictions for milk spoilage risk assessment and management.

P2-42 Hyperspectral Imaging for Rapid Identification of Foodborne Pathogens at the Colony Level

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❖ ***Developing Scientist Entrant***

Introduction: Hyperspectral imaging (HSI) integrates conventional imaging and spectroscopic techniques to simultaneously acquire spatial and spectral information. The HSI is a novel optical tool in food processing that has a great potential for rapidly identifying bacterial colonies on agar media.

Purpose: The aim of this study was to obtain hyperspectral data of bacterial colonies isolated from pure cultures to build a reference library and subsequently evaluating the accuracy of HSI setup to classify bacteria isolated from artificially inoculated food matrices (milk or milk powder).

Methods: This study was designed as a randomized complete block design with six replications. Three strains of *Listeriamonocytogenes* (LM), 4-strains of *Escherichia coli O157: H7* (EC), big six non O157:H7 EC, 3-strains of *Staphylococcus aureus* (SA), and 10-serovars of *Salmonella* (SAL) were used in this study. Pure cultures were streaked for isolation on respective selective media. Milk or whole milk powder were artificially inoculated with individual pathogenic strains or serovars (<10 CFU g or mL). Before isolation, the respective samples were enriched using Brain Heart Infusion (BHI) broth at 37°C for 24 hrs. For an individual pathogen, the isolated colonies on a petri dish were analyzed under the HSI setup to gather hyperspectral data. The acquired images were imported into ENVI software, and 3 regions of interest (ROI) were selected for each image.

Poster

Results: Using *k*NN classifier and cross validation technique, an accuracy of 97.2, 97.2, 96.6, and 75.8 % were obtained for LM, SA, SAL, and EC, respectively, at genus level. The classification accuracy of LM and SA strains varied from 83.33 to 100%. Whereas, all serovars of SAL, except Typhi had classification accuracy of 100%. The classification accuracy of EC strains varied from 16.67 to 100%.

Significance: Future work will be done to increase the size of reference library and shortening the enrichment time.

P2-43 Assessment of Date Fruit-Flavored Drinkable Yogurt

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Introduction: Drinkable yogurt with fruit flavors and probiotics is currently prevalent. Date fruit powder is high in nutrients which could be used as natural source of dietary fiber in yogurt drinks and stirred yogurts.

Purpose: The aim of this study was to examine the microbiological, physicochemical, and rheological properties of drinkable yogurt.

Methods: As a control sample, fresh milk was inoculated with (1.3 g/L, w/v %) starter cultures "Y5 culture". fortified with different concentrations (0.4,0.8, 1.0, and 1.5 %) of date fruit powder (DFP) for 15 days storage at 5-7°C.

Results: Our results indicated that the pH values of the control and other DFP treatments (4.8, 4.86, 4.62, 4.55, and 4.58) were slightly decreased to (4.57, 4.66, 4.59, 4.54, and 4.56, respectively) during the storage period. Microbiological examination revealed that the initial yogurt culture counts (8.73±0.01, 8.70±0.02, 8.80±0.6, 8.83±0.6, and 8.34±0.01 Log CFU/mL) in fortified samples were significantly increased as DFP ratio increased and decreased (7.96±0.01,8.00±0.03, 7.31±0.01, 7.18±0.01, and 6.25±0.05 Log CFU/mL) as the storage period progressed compared to the control sample (without DFP). The rheological study displayed shear thinning behavior on the control and fortified drinkable yogurt samples. The samples fortified with DFP showed an increase in viscosity (96, 266, 346, and 141 Pa. s, respectively) compared to the control 27 Pa. s. After 15 days of storage, the samples fortified with 0.4 and 1% of DFP has the highest increase in viscosity 282 and 1162 Pa. s, respectively compared to the control 16.7 Pa.s. Our results showed that adding DFP significantly increased the redness parameter (a*) and decreased the lightness parameter (L*). The samples fortified with 1.5% of DFP showed the highest a* (1.31±0.16) and lowest L* (83.85±.0.09).

Significance: Our findings suggest that DFP could improve the viability, acidity, the physicochemical and functional properties of the flavored drinkable yogurt.

P2-44 Finding Needles in Haystacks: Detection of Foodborne Pathogens When Sampling Volume and Prevalence Are Low

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Introduction: Addressing food safety in sub-Saharan Africa is fraught with many difficulties such as limited funding for extensive sampling and low detection probabilities of foodborne pathogens.

Purpose: Here, we demonstrate how to overcome such difficulties with a pooled sampling design that is illustrated via a case study of *Salmonella* in tomatoes sold at markets in sub-Saharan Africa.

Methods: A literature survey of *Salmonella* in tomatoes at African markets indicated a prevalence range between 0.00-0.37%. These estimates served as the basis to address two statistical questions. (1) What is the probability of a *Salmonella* positive pool in relation to the prevalence estimate per tomato? and (2) What is the probability that a positive pool is caused by exactly 1 contaminated tomato?

Results: Our results indicated that at a low *Salmonella* prevalence even large pool sizes result in low detection probabilities. However, when prevalence increases to at least 0.1% the probability of finding a positive pool increases sharply. For example, if the prevalence is 0.32% at a pool size of 30, the probability of a pool being positive pool is 9.20%. At a prevalence of 0.32% the probability of a single positive tomato in a pool of 30 is 95.4%. This indicates that pool sizes of 30 tomatoes are optimal for calculating individual level prevalence of tomatoes in African markets.

Significance: In subsequent steps, the effects of the number of pools on prevalence estimates, transitioning from prevalence of sample pools to individual-level prevalence and, power to detect prevalence differences in treatment types can be accounted for. The methods presented here enable prevalence estimation when sampling is limited, and pathogens are difficult to detect.

P2-45 Foodcontroller: An R Package for Automatic Development of Machine Learning Models for Predicting Quality of Meat Products

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Introduction: Monitoring microbial activity and measuring biochemical changes occurring in food is the key point to evaluate freshness of food and quality. Several rapid, non-destructive analytical methods such as multi-and hyper-spectral imaging techniques are gaining popularity over inadequate conventional and expensive microbial techniques. The downside of the analytical platforms is the considerable size and complexity of data generated by such methods. Therefore, statistical and machine learning algorithms are usually applied to interpret the outcome. However, there are no certain rules about which machine learning methods should be applied to which analytical platform to ensure the best prediction outcome.

Purpose: In order to overcome the difficulties, we developed FoodQualityController, a flexible and user-friendly R package that automate the process of identifying, validating, and optimizing the most suitable machine learning platform for the given analytical platform.

Methods: Several machine learning algorithms are implemented within the package in order to optimize the process for predicting microbial quality in beef products.

Results: In this work, a new user-friendly, flexible, standalone R package named 'FoodQualityController' is created. (https://github.com/ozlemkaradeniz/FoodQualityController) The package runs several novel machine learning models such as SVM, Neural Network, RandomForest, XGBoost, KNN, PLS, PCR to predict microbial quality in food products and produces statistical results for the user. The models are trained and tested with datasets produced by various analytical platforms such as FTIR, enose, VideometerLab, VideometerLabUV and Fresh Detect.

Significance: Overall, the study proves that computational methods could be promising rivals to the traditional food quality assessment methods. FoodQualityController has produced outstanding prediction accuracies which could make computational techniques take over conventional molecular techniques in the future. Finding the best fitted combination of ML algorithm and analytical platform by FoodQualityController and receiving the quality results straightaway in a few minutes could create a more efficient product chain.

P2-46 Data-Mining Poultry Processing Bio-Mapping Counts of Pathogens and Indicator Organisms for Food Safety Management Decision Making

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Introduction: Bio-mapping studies play an important role as data collected can be managed and analyzed in multiple ways to look at process trends, find explanations about the effect of processing and intervention changes, and even compiling microbial performance data to demonstrate the effect of certain decisions taken on a daily basis and their effects overtime in commercial settings to inspection authorities.

Purpose: The overall goal was to show different perspectives for data analysis by taking advantage of a comprehensive bio-mapping study conducted in a poultry processing facility to demonstrate how this analysis can be used by the processor for making decisions that are sound, robust, and supported by comprehensive information of the process.

Methods: Nine locations throughout a poultry processing facility were sampled including: live receiving, rehanger, post-eviscerator, post-cropper, post-neck-breaker, inside-outside-bird-wash, pre-chill, post-chill, and wings at reduced and normal chemical intervention levels (100 to 400ppm of peroxyacetic acid and 50ppm of total chlorine). A total of 800 whole carcass/part rinsates were collected during a seven-month period on different days and shifts. Aerobic counts, Enterobacteriaceae, *Salmonella* and *Campylobacter* counts were determined and analyzed using R to evaluate differences in shifts, correlation between indicators and pathogens counts, and distribution analysis for all locations.

Results: A greater number of locations were statistically different between shifts under reduced levels of chemical interventions with higher means at the second shift for both indicators and pathogens levels (P=0.02). Minimal to negligible correlation was found when comparing aerobic counts and Enterobacteriaceae counts with *Salmonella* levels, with significant variability between sampling locations. Distribution analysis and visualization as a bio-map of the process resulted in a clear bimodality in reduced chemical conditions for multiple locations mostly explained by shift effect.

Significance: The development, use and alternative analysis of bio-mapping data, including proper data visualization, improves the tools needed for ongoing decision making in food safety systems.

P2-47 Data Analytics and Management in an Italian Olive Oil Farm 4.0: A Case Study from Umbria Region, the Green Heart of Italy

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Introduction: One of the biggest challenges of the twenty-first century is to produce more food, using fewer resources (inputs), while respecting the environment.

Purpose: The study aims to combine productivity and sustainability by introducing new tools replacing traditional farming methods with innovative ones, rationalizing water, insecticides, and fertilizers, using as needed.

Methods: The study focused on olives trees planted in 2018 on volcanic and calcareous-silicic soil originated in a plot of 300 ha with a traditional planting layout 6x6 meters using cultivars of European Olea "*Leccino*", "*Moraiolo", "Frantoio", "Coratina", "Leccio del Corno",* and innovative planting layout "Espalier breeding" with cultivar of *European Olea "Favolosa"* (FS17), *"Lecciana", "Arbequina", "Manzanilla", "Coratina", "Nocellara del Belice", "Oliana*" and *"Don Carlo".* The planthas adopted the agronomic method of mulching, the hydraulic system of sub-irrigation monitored by microprobes, GSP geolocation system with RTK correction method and finally the use of renewable energy from photovoltaics.

Results: Mulching reduces weeds, promotes growth, and maintains the favorable temperature by removing the damage caused by the cold; the GSP-RTK system analyzed the soil three-dimensionally to rationally optimize the spaces layout. Subirrigation allows a precise distribution of water and fertilizers directly in the area explored by the root system optimizing the used substances; thanks to the probes, the field water capacity is kept under control, acting at the appropriate time.

Significance: This case study combines agrotechnical, physical, and chemical principles and different advanced technologies to achieve the goal of a full environmental and power sustainability, optimizing the use of energy, water and fertilizers resources in both traditional and intensive innovative planting layout.

P2-48 Exploring the Ethical Implications of Artificial Intelligence in the Food Safety Field

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Introduction: In the food safety field, Artificial Intelligence (AI), big data, and cloud computing are recently being explored and applied to food safety prediction, risk assessment, disease surveillance, and food quality evaluation. As with any emerging technology, ethical concerns are bound to arise.

Purpose: This work seeks to encourage conversations on the role of food safety professionals in addressing ethical issues that may be associated with the use of AI in the food safety field.

Methods: A search of scientific literature was performed in Web of Science and Google Scholar using terms such as "AI ethics", "AI food science", "AI food safety", "ethical implications of AI", "ethical issues AI", and "AI impacts". A comprehensive review of selected research papers was performed to identify the research areas and ethical themes in these publications.

Results: AI ethics has been widely studied by researchers from fields such as computer science, humanities, policy, and medicine. However, there has been a low number of publications from researchers in the food safety field. This could be attributed to the relative newness of AI usage in food science as a whole and the lack of ethical analysis expertise among food scientists. There are concerns about whether to hold an AI solutions provider or AI user (e.g., food company) responsible if an AI-enabled decision were to result in a foodborne disease outbreak. There are also concerns about the rights and control that AI solutions providers and users should have over data generated in the users' facilities. Furthermore, ethical themes such as bias, privacy, transparency, and economic and environmental impact, have been raised concerning the application of AI in food safety.

Significance: AI applications in the food safety field are increasing rapidly. Food safety professionals are better placed to understand the field and the unique ethical issues that may arise. It is imperative that food safety professionals lead, contribute, and own the conversation on AI ethics in their field.

P2-49 The Journey Towards Modernizing the Publicly Available International Foodborne Outbreak Database

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Introduction: The Publicly Available International Foodborne Outbreak Database (PAIFOD) has been systematically collecting international foodborne outbreak data from publicly available sources since the year 2000 to respond to stakeholder requests.

Purpose: A major modernization effort was undertaken to change technology, realign PAIFOD's structure with the current food safety landscape, terminology, and data requirements, thus increasing the ease of reporting on and analyzing foodborne outbreaks.

Methods: Database technology challenges and opportunities for improvement of the structure and organization of data were identified. A combination of formative research, discussion with a team of food safety epidemiologists and risk assessment specialists as well as some external consultations were conducted to map out a database restructure onto a new platform.

Results: The database was transferred from Microsoft Access to an open-source software, Baserow.io which solves many limitations of the Access database including allowing for multiple selections within categories and the ability to catalogue many sources of outbreak information. All listed food vehicles were re-grouped into higher level categories to mimic the Interagency Food Safety Analytics Collaboration (IFSAC) food category structure, allowing for analysis of larger categories of food and more efficient reporting. A user-friendly structure was established to accurately report details of microorganisms down to serovar. Data tags were developed to indicate the availability of antimicrobial information, popular food attributes such as frozen, fermented, or imported, and outbreak investigation methods. This presentation will walk through the new database framework and discuss the challenges and solutions to modernizing this 20-year-old database.

Significance: Changes in food safety surveillance, foods consumed, emerging risks in the past decades, and changes in technology necessitated the modernization of the PAIFOD database to continue to support our Canadian and international clients with summaries and analyses of trends in international foodborne outbreaks.

P2-50 Optimization of DNA Extraction and Amplification Protocols to Improve Accuracy of Plant Species Identification by DNA Metabarcoding

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Introduction: DNA metabarcoding is a Next Generation Sequencing (NGS) method that can be used to identify the components of mixed samples to genus or species level. This approach is a powerful tool for the detection of food fraud or adulteration in plant or botanical ingredients.

Purpose: The objectives of this work are to optimize the DNA extraction, PCR amplification, and NGS library preparation steps for a plant metabarcoding protocol.

Methods: Individual samples of plants (thyme, nutmeg, cayenne, corn) along with a mix of spices (9% thyme, 90% nutmeg, 1% cayenne) underwent DNA extraction using both a CTAB method and a mechanical lysis method. DNA was quantified and used to generate amplicons for five sequencing targets: *matk*, *rbcLa*, *trnL*, ITS2, and *psbA/trnH2* intergenic spacer region. Two sets of primers, with and without adapters, were used to amplify the targets. PCR products were visualized by gel electrophoresis, then used as template for sequencing on the Illumina MiSeq.

Results: There was improved amplification with the use of non-adapted primers for corn, nutmeg, and thyme (additional targets amplified). With mechanical lysis, DNA yield was significantly increased for thyme, cayenne, and spice mix ($P<0.05$ when analyzed using a paired t-test). When comparing chemical (CTAB) and mechanical lysis extraction, results were similar although thyme showed improvement with mechanical lysis (5 PCR products vs. 3 with CTAB).

Significance: This work identifies a number of method improvements that increase the quality of plant DNA metabarcoding results. These improvements led to an increase in classified reads and the ability of the method to distinguish minor components in a sample.

P2-51 A Green Analytical Method for Fish Species Authentication Based on Raman Spectroscopy

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Introduction: Fish mislabeling is a rampant global issue, damaging consumers' economic benefits and trust in the fish industry and government authorities, as well as diminishing the efficacy of the sustainability measurement and management of fisheries.

Purpose: This study aimed to develop a rapid, easy-to-use, and environmentally-friendly method for fish species identification using non-destructive Raman spectroscopy with chemometrics/machine learning algorithms.

Methods: A portable Raman spectrometer and a benchtop confocal Raman spectrometer were used and compared for their performance to identify 11 species of fish (*i.e.*, 4 species of Salmonidae and 7 species of non-Salmonidae). Supervised chemometric/machine learning classification models were constructed based on a hierarchical classification principle to solve this 11-class identification problem.

Results: Both Raman spectrometers were able to differentiate Salmonidae from non-Salmonidae fish with close to 100% accuracy (*i.e.*, first hierarchy level). To further identify the fish to species level, the portable Raman spectrometer provided better accuracy (*i.e.,* 93% accuracy for both the Salmonidae and non-Salmonidae group of fish) compared to the benchtop Raman spectrometer (*i.e.,* 90% and 84% accuracy for the Salmonidae and non-Salmonidae, respectively). The overall analysis from sample to results was completed within 5 min, much faster compared to the gold standard DNA barcoding method. The classification power of this Raman spectroscopy-based technique is expected to be improved with an increased spectral number of fish species and biological replicates in the Raman spectral library, as well with advanced machine learning algorithms.

Significance: This rapid and reliable fish authentication method based on Raman spectroscopy will provide government laboratories and the fish industry another useful tool to routinely and frequently monitor the fish authenticity, and thus to protect consumers' benefits and guarantee the efficacy of the fishery sustainability measurement and management strategies.

P2-52 Chemical and Genetic Variability of Four *Cinnamomum* Species for Food Safety Applications

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❖ ***Developing Scientist Entrant***

Introduction: Cinnamon (*Cinnamomum verum*) is widely used in various plant-based food products. However, it is highly susceptible to adulteration with the morphologically similar, cheaper, and hepatotoxic species in global markets.

Purpose: To determine chemical and molecular markers for discrimination of *C. verum* from its adulterant species.

Methods: Samples of the four *Cinnamomum* species (*C. verum*, *C. loureiroi*, *C. burmannii*, and *C. cassia*) were collected. The 80% methanolic extracts were subjected to GC-MS (gas chromatography–mass spectrometry) analysis for the characterization of chemical compounds. To study the genetic diversity among the four *Cinnamomum* species, the nucleotide sequences of the plastid (*rbcL* and *MatK*) and nuclear (ITS1 and ITS2) regions were collected from the NCBI database. The phylogenetic trees were constructed by the neighbor-joining (NJ) method. Genetic distances were calculated using the Tamura-Nei method. Finally, the target region was selected to design a *C. verum*-specific primer.

Results: C*. verum* contained the highest eugenol (5.77%) and the lowest coumarin (1.90%) than the other three species. The sequence divergence in the nuclear regions (22 to 37%) was higher than one in the plastid regions (1 to 3%). Moreover, the ITS2 region presented a higher genetic distance between *C. verum* and its adulterant species compared to the ITS1 region. The specifically designed primer based on the ITS2-26S region showed positive amplification only for *C. verum*. PCR-sequenced product showed 100% homology with the consensus sequence.

Significance: A combination of chemical and molecular markers could be potential tools in the identification of *C. verum* for food quality and safety purposes.

Poster

P2-53 Authenticity of Plant-Based Products Sold in Piracicaba – SP City

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❖ ***Developing Scientist Entrant***

Introduction: Plant-based foods have become the target of studies to be an alternative source of protein in the population's diet, and the regulation lack in this market allows the occurrence of failures in processing and food fraud.

Purpose: The aim of this study was to report results on the authenticity of plant-based food products collected in supermarkets located in the city of Piracicaba, SP – Brazil.

Methods: A total of 25 samples analogue to hamburgers (n=9), ground beef (n=1), shredded beef (n=1), meatballs (n=1), loin (n=1), kibbeh (n=3), sausage (n=1), breaded chicken (n=2), fish ball (n=1), dairy beverage (n=4), and cheese (n=1) were collected from two supermarkets located in Piracicaba - SP city. Samples were submitted to DNA extraction using InstaGene™ Matrix and to conventional polymerase chain reaction (PCR) method to identify the presence of animal species (pork, cattle, chicken, and fish) and soy undeclared.

Results: Only one of the 25 samples had a declaration of the possibility of containing unintentional animal DNA, as it had been processed in the same animal products processing plant. In addition, soy, and its by-products (oil, isolated protein, and textured soy protein) were declared on nine plant-based products labels. In this study, the plant-based products sold in Piracicaba-SP city evaluated were considered authentic according to the method and parameters used, since no undeclared animal and soy DNA was detected.

Significance: These results can contribute to the safety actions establishment in the food processing industries and inform consumers about plant-based food consumed authenticity.

P2-54 Geographical Origin Discrimination and Quality Evaluation of Oolong Tea Using Color Analysis and Electronic Nose Coupled with Chemometrics

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❖ ***Developing Scientist Entrant***

Introduction: Taiwan produces one of the finest qualities of oolong tea in the world. However, because Taiwanese tea production is insufficient to meet demand, fake oolong tea imported from Vietnam and China is sold as Taiwanese oolong tea at inflated prices, affecting both customer satisfaction and tea companies' profits.

Purpose: To conduct color analysis and further investigate the ability of the electronic nose (E-nose) to discriminate the quality of oolong tea using chemometrics.

Methods: 60 oolong tea samples (3 groups of 20 samples each) were collected from three different geographical origins (Taiwan, Vietnam, and China). The color parameters (L*, a*, and b*) were evaluated. For E-nose analysis, a 5 g tea leaf sample in a beaker was sealed with plastic wrap to generate headspace gas. The pumped gas reacted with 14 different sensors, and the change in electric conductivity was measured as each sensor's response signal. Multivariate analysis of variance (MANOVA) was performed to determine the significant interactions between volatile components and tea origin. Chemometric methods, including principal component analysis (PCA) and discriminant analysis (DA), were employed for the classification of tea samples. Each sample was analyzed in triplicate.

Results: L*, a*, and b* color values significantly differed (P<0.05) between tea samples. MANOVA also showed statistically significant differences in the volatile components of tea samples based on origin, F (28, 328) = 237.93, P< 0.001; Wilk's Λ = 0.002, partial η^2 = 0.95. The first three extracted PCs (PC1=50.24%, PC2=26.18%, and PC3=13.08%) contributed to 89.50% of the cumulative variance. PCA score plot showed satisfactory separation of tea samples according to origin. The correct classification rate of tea samples achieved by DA was 100%.

Significance: An E-nose-chemometrics approach could be used as a rapid tool for discriminating the quality of oolong tea from different geographical origins.

P2-55 Food Integrity Climate and Culture Assessment in Food Businesses

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Introduction: Understanding food fraud and food integrity within different types of food businesses, identifying their key factors and developing assessment tools and strategies contribute to fostering food integrity.

Purpose: This research explores the association of food integrity culture in food processing organizations.

Methods: The prevailing food integrity culture of four Belgian food companies was assessed through a method triangulation: the relation between the perceived food integrity climate (self-assessment of all employees), the performed food integrity (compliance-based observation of product, process, people and data) and the companies' food fraud vulnerability was analyzed in view of employees and organizational characteristics.

Results: All the participating companies recorded a positive food integrity culture, since their food integrity climate and food integrity performance were medium-high and their food fraud vulnerability was medium-low. People integrity was the lowest-perceived dimension and specific food fraud control measures were found to be missing. Managers perceived their company's food integrity climate higher than the operators in contact with food, implying that employees in different job functions or roles may hold differing perceptions of their company's food integrity climate. Results suggest that product type, company size and certifications status may also promote (or hinder) the achievement of a positive food integrity culture.

Significance: The proposed food integrity culture method triangulation has demonstrated to assist food companies in acknowledging potential weaknesses in their food integrity climate, food integrity performance and food fraud control measures, allowing them to improve key human, operational, technical and managerial aspects to achieve an overall consolidated food integrity culture.

P2-56 Multi-Signal Forecasting for Food Fraud: A Case Study on the Beef Supply Chain

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Introduction: A global trans-national issue which affects both consumers and the agri-food sector is food fraud. To address this issue, AI models combining various indicators can be developed, which can act as signals for the timely forecasting.

Purpose: We developed a general methodology for the identification of important signals, the collection of relevant data sources, the cleaning of data records, and their incorporation in state-of-the-art AI forecasting models. Thus, the main objective of the study was to predict beef fraud incidents by means of AI technology.

Methods: The methodology has been applied on the beef supply chain targeting the timely forecasting of fraud incidents. Through extensive literature review and coordination with stakeholders and food industries in the relevant supply chains, we identified multiple signals and data sources including authenticity test results, historical fraud incidents, economic, environmental, and governmental indicators of sourcing countries/suppliers. In all cases, both public and private data records were analyzed. The forecasting AI model family used was based on multivariate time series forecasting.

Results: The predictive model, which is trained and validated, shows a variety of emerging risks in the beef supply chain. Specifically, as geographical origin of potential fraud incidents, Uruguay and Brazil were highlighted. Factors which affected the model's prediction the most, were the ones involving the global beef trade (production, trade, and disease data).

Significance: Our study shows that multi-signal forecasting can provide validated information about potential future beef fraud incidents. Implementation of this model in the food industry as an early-warning prediction tool of such incidents could enhance existing food safety management systems and consumer's satisfaction, as well. The relevant trained AI model has been incorporated as a commercial solution in our FOODAKAI food intelligence application and is currently in use at relevant food industries.

P2-57 Development of an Intact Protein Mass Spectrometry Method for Milk Authentication

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❖ ***Developing Scientist Entrant***

Introduction: Authentication of products with claims regarding protein sources or compositions is a challenge for traditional analytical methods, which generally lack the required specificity. For example, establishment of milk as "A2" is done through genetic testing of cows before milk production, with no methods to authenticate milk products themselves.

Purpose: This study aimed to develop an intact protein MS method to analyze major bovine milk proteins in powdered and high-temperature, short-time (HTST) milk for direct authentication of protein products, including specific proteoform claims.

Methods: Informatics approaches were employed to generate a database of theoretical monoisotopic masses of all major milk protein proteoforms. Powdered and HTST milk samples were diluted (1 mg milk protein.ml^{-1}), defatted through centrifugation, and desalted using molecular weight cutoff spin filters. Sample protein was separated and analyzed by UHPLC-MS. Data was deconvoluted to generate a list of monoisotopic masses. Mass events of 6-27 kDa were analyzed against the predicted milk protein mass database (mass error tolerance = 10 ppm).

Results: A mean of 94.6% (± 2.8%) (n=8) of the total signal of powdered milk could be assigned to predicted database proteoforms. Multiple lactosylated proteoforms of β-lactoglobulin (A and B) were identified, with a consistent ratio of 0.41 (± 0.01): 0.41 (± 0.01): 0.18 (± 0.02) (0: +1: +2 lactose adducts). Similarly, A2 β-casein showed a consistent ratio of 0.80 (± 0.03): 0.20 (± 0.03) (0: +1 lactose adduct). Ratio monitoring and signal comparison of these two proteins may allow for authentication of A2 milk.

Significance: This work demonstrates that intact mass spectrometry can be used to analyze milk products for protein authentication.

P2-58 OFAS Pre-Market Review Programs: An Introduction to GRAS and the GRAS Notification Program

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Introduction: Evaluation of ingredients added to human food is an important element of food safety, helping to ensure the safety of ingredients prior to marketing products to consumers; however, the regulatory standards in the U.S. are robust, relying on multiple elements to reach a conclusion of safe use.

Purpose: This presentation seeks to elucidate one element of food ingredient regulation in the U.S., generally recognized as safe (GRAS), as it is often misunderstood.

Methods: In the U.S., food additives are substances that are intentionally added to food and are subject to pre-market approval by the Food and Drug Administration (FDA) unless the use of the substance is GRAS, among qualified experts, for its intended use. This is referred to as the GRAS provision. GRAS is not an inherent property of a substance, as is often misunderstood; instead, it relies on the substance's proposed use and use level, the manufacturing process, dietary exposure, and other relevant safety data and information. FDA operates a notification program whereby a manufacturer may choose to inform FDA of its conclusion that the use of a substance is GRAS.

Results: Since the inception of the GRAS Notification Program, FDA has filed more than 1000 GRAS notices (GRNs). Manufacturers of novel ingredients, including "alternative" proteins, bacteriophage preparations, enzymes, and human milk oligosaccharides, have submitted dossiers through the program. For a GRN to be filed and evaluated by FDA, the submission must meet the requirements stipulated in the Code of Federal Regulations, including containing sufficient identity, manufacturing, and safety data and information in support of a GRAS conclusion.

Significance: Food ingredient regulation in the U.S. is complex; however, FDA routinely engages in outreach to help food safety professionals understand the GRAS requirements and make a robust GRAS conclusion, ensuring safety of the food supply.

P2-59 Quantification of Beef in Products Sold in Canada Declaring Multiple Meat Species – Regulatory and Consumer Implications Related to Accurate Labeling

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❖ ***Developing Scientist Entrant***

Introduction: Unlike other jurisdictions (i.e., European union), Canadian regulations do not require the declaration of the percentage of each meat species present in a prepackaged food product. Standards of identity establish total minimum meat protein contents for different categories, but declaration is required only for certain products to which phosphate salts and/or water are incorporated. In addition, "and/or" may be used to indicate the potential presence of a given meat species in the formulation.

Purpose: The purpose of this study was to determine the beef content of processed mixed meat products sold in Canada with labels mentioning (i) beef, and (ii) "and/or" beef.

Methods: Beef DNA in 69 products declaring beef on their labels (e.g., frozen burgers, sausages, canned meatballs) and in 12 products declaring "and/or" beef (e.g., pepperoni, hot dogs, meat-based sauce) was determined using qPCR (SureFood® ANIMAL QUANT Beef kit, R-Biopharm; MIC thermocycler), analyzed in duplicates. Beef content was expressed as a percentage of the total meat present in the food product by calculating the ratio of beef DNA copies/total DNA copies obtained with the kit's standard curve.

Results: Among products declaring beef, 9 had no detectable beef DNA (<0.04 %, the kit's limit of the detection), 9 were considered to contain only traces (0.45 % +/- 0.32) and 51 contained > 1 % beef. Among products declaring "and/or" beef, 4 had no detectable beef DNA (< 0.04%) and 8 contained only traces (0.31 % +/- 0.32).

Significance: These results suggest that the declaration of exact meat species content in prepackaged meat products could enhance transparency and better meet Canadian consumers' expectations. This hypothesis will be further evaluated through the analysis of additional samples and the development of a consumer survey (in preparation).

P2-60 Front-of-Pack Nutrition Labelling: Global Outlook

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Introduction: Front-of-pack (FOP) nutrition labelling is among a suite of public health tools available to policymakers and regulators to combat obesity and diet-related non-communicable diseases (NCDs): from LATAM to Europe, from Canada to Middle East, India and Australia, public health agencies have proposed forms of FOP labelling to communicate nutrition values more effectively to consumers. NCDs are intrinsically complex and multifactorial, often

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driven by stress, lack of time, limited budget, decision-fatigue and not necessarily by lack of information. Regulators have decided that the clear, immediate, and balanced expression of the main nutrients on the front-of-pack can help consumers to make better choices.

Purpose: If the answer is FOP, the question we're left with is: what should FOP look like? To date, most countries that have FOP frameworks have created new ones. The multiplicity of FOP that have been proposed or enacted is raising the issue of the harmonization for industry and for consumers. In a global society, how can a consumer understand tens of different labelling schemes? And are these schemes damaging to international trade? Is it possible that certain schemes might arbitrarily discriminate against certain food categories?

Methods: The fight between colour-coded schemes and less suggestive ones is particularly harsh in Europe, where the EU Commission intended to propose new legislation by the end of 2022. Powerful countries are sponsoring different options. In Canada, a new scheme will be mandatory by 1st January 2026. The speakers will offer insights on the problems and opportunities with different schemes and practical comparative examples.

Results: The objective of the panel is to summarize the status quo and analyse possible ways forward.

Significance: This panel and related outcomes will be of interest to anyone working in public health and safety, food law, regulatory compliance, and industry.

P2-61 Health Canada's Updated "Policy on *Listeria monocytogenes* in Ready-to-Eat Foods" (2023)

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Introduction: Health Canada, a federal governmental institution, has the safety of the Canadian food supply at the top of its priorities. As such, it continuously reviews existing policies to protect the health and safety of Canadian consumers by minimizing public health risks from the consumption of foods that contain microbial pathogens.

Purpose: The updated *Listeria* policy (2023) guides industry on ways to comply with federal food legislation and may be used as a resource by regulatory authorities for enforcement. Hence, potentially unsafe ready-to-eat foods are more likely to be discovered before they hit the store shelves.

Methods: As part of the periodic review process, which included two public consultations with stakeholders, Health Canada examined the latest science, considered the evolving Canadian food environment and regulatory landscape, and explored ways to improve guidance related to the application, implementation and verification of control measures for *Listeria monocytogenes* in ready-to-eat foods to food businesses and regulatory authorities.

Results: These consultations allowed Health Canada to collect information, suggestions and diverse perspectives from users of the *Listeria* policy which supplemented a thorough literature review of the latest science for the update of the *Listeria* policy. Four key themes emerged during the periodic review:

1. overall clarity of concepts and predictability
2. roles and responsibilities of government, industry and consumers
3. control measures for *L. monocytogenes*
4. ready-to-eat foods specifically produced for consumption by vulnerable populations

The *Listeria* policy was updated while keeping its risk-based principles: ready-to-eat foods continue to be categorized based on their potential to support *L. monocytogenes* growth; focus is given to environmental control and monitoring; and sampling guidelines for environmental monitoring remain risk-based.

Significance: The *Listeria* policy (2023) provides clear, effective and predictable guidance that is science- and risk-based, and that is better adapted to the current Canadian food environment.

P2-62 Can Food Safety Assessment Tools Correlate with COVID-19 Protocols?

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Introduction: Checklists are used during inspections to assess the hygiene and food safety performance of food services, especially during the pandemic period.

Purpose: To evaluate the correlation between the measures from the food safety assessment tools and the COVID-19 health protocol for food services.

Methods: Forty food services were inspected, and four checklists were applied to assess i. the level of risk for foodborne diseases (FBD), ii. good handling practices, iii. infrastructure, and iv. implementation of the COVID-19 health protocol. The risk assessment tool was interpreted using a risk score, with each item weighted according to the risk of an FBD. The results of the other tools were evaluated using an overall percentage of violated items. Pearson's correlation coefficient was used to evaluate the correlation between food safety assessment scores and compliance with the COVID-19 health protocol. The study was approved by the Research Ethics Committee under number 4.666.270.

Results: Food services establishments were inspected, 90% of which had a very high risk of FBD. Regarding the COVID-19 protocol, 35% of the establishments presented a moderate percentage of violations. Regarding the percentage of violations of good practices and infrastructure, it was found that 60% and 45% of the establishments presented a high percentage of non-compliance, respectively. The degree of risk showed a strong positive correlation with the percentage of good handling practice violation ($r=0.87$; $p<0.001$) and percentage of infrastructure violation ($r=0.75$; $p<0.001$). Regarding the instrument for evaluating the implementation of the COVID-19 protocol, a moderate correlation was found with the good handling practices violation percentage ($r=0.60$; $p<0.001$).

Significance: A significant correlation was found between the food safety assessment tools measures and the COVID-19 health protocol for food services. Discussion of the relationship between the instruments may help develop checklists to standardize health inspection activities and reduce the risk of FBD.

P2-63 Comparison of the Efficacy of Different Organic Acid Salts Against *Listeria monocytogenes* in a Low Sodium Hot Dog Formulation

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Introduction: Given the market trend of sodium reduction, potassium-based solutions are preferred antimicrobials. In the present work, potassium acetate/diacetate-blend is evaluated on antimicrobial efficacy against L. monocytogenes on hot dogs.

Purpose: To assess the antilisterial effects of potassium lactate, sodium acetate and potassium acetate/diacetate (Provian K) in frankfurters stored at 4 and 7°C for 120 days.

Methods: A total of 2000 hot dogs for different treatment formulations (0.25-0.75% Provian K, 0.06-0.16% sodium acetate and 1.17-3.25% potassium lactate) and control (no antimicrobials) were inoculated in-pairs with five-strain cocktail of *L. monocytogenes*. Following bacterial attachment, the hot dogs were vacuum packaged and stored at 4 and 7°C and sampled up to 120 days. At each sampling, hot dogs were homogenized using a stomacher and plated onto Modified Oxford medium for enumeration. Failure of antilisterial capacity was assessed at 2 log CFU/g outgrowth, and treatment performance was compared using one-way ANOVA ($p<0.05$). The enumeration data was fitted to modified Gompertz model to calculate the lag time (day) and maximum growth rate (μmax; log/day) for each treatment.

Results: Inoculation level of 2.5 log CFU/g of *L. monocytogenes* was achieved on day 0 for all the treatments. The control treatment exhibited fastest outgrowth (>2 log CFU/g) of *L. monocytogenes* by 21 days and 3 days of storage at 4 and 7°C, respectively. The 0.75% Provian K treatment significantly ($p<0.05$) controlled *L. monocytogenes* outgrowth (<2 log CFU/g) > 63 days and 7 days of storage at 4°C and 7°C, respectively. At 4°C, Provian K exhibited highest lag

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time and slowest µmax of (20 day; 0.05 log/day) compared to control (9 day; 0.13 log/day) while at 7°C, Provian K and sodium acetate treatments exhibited significantly ($p<0.05$) reduced µmax compared to control and potassium lactate treatments.

Significance: Potassium acetates/diacetates can be used as highly effective alternatives to classic lactate-based *Listeria* interventions.

P2-64 Development and Evaluation of a Digital Storytelling to Improve Food Safety Behavior Change

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Introduction: Traditional food safety training and education approaches are effective to improve food handler knowledge level but their impact on changing food handler attitudes and behavior change is less consistent.

Purpose: This study was undertaken to develop and evaluate a digital storytelling video as an innovative and engaging approach to enhance the effectiveness of education and training strategies in the ready-to-eat (RTE) meat processing sector.

Methods: A digital storytelling video was developed based on semi-structured in-depth interviews with RTE meat processing plants in Ontario to highlight success factors and how to overcome any barriers or challenges to implement food safety practices in the meat processing sector. A preliminary descriptive evaluation of the storytelling video was also conducted through an online survey with meat processing plants and other industry stakeholders about the applicability and accessibility of the content and format, and their potential adoption and implementation of learned content.

Results: The storytelling video covered important food safety practices (e.g., sanitation, temperature control, pre-operational inspection, and pest control), difficulties and challenges (e.g., capital and labor), solutions and opportunities (e.g., high quality and safety standards for products, strong food safety culture, effective communication, and employee engagement opportunities), and food safety training resources. By learning from their peers through the storytelling video, RTE meat plants would be able to understand how they can practically implement food safety practices using available and existing resources and strategies within the industry. The storytelling video seems to be an effective knowledge translation and transfer tool to achieve enhanced behavior change and improve food safety practices.

Significance: The storytelling video developed in this study can help to promote and encourage more effective food safety behavior change and adoption of food safety practices in the RTE meat processing sector to reduce the risk of foodborne illness associated with RTE meats.

P2-65 Factors Influencing Successful Implementation of Food Safety and Good Manufacturing Practices in Ready-to-Eat Meat Processing Plants in Ontario, Canada

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Introduction: Food safety inspections of ready-to-eat (RTE) meat processing plants have identified a lack of compliance with a good manufacturing practices (GMPs), indicating that there is a need to improve food safety practices to reduce food safety risks.

Purpose: This study was undertaken to identify the key factors for the successful and practical implementation of food safety practices and GMPs using a qualitative interviewing approach.

Methods: Semi-structured in-depth interviews and open-ended online survey were conducted with RTE meat processing plant operators in Ontario, Canada. Data were analyzed descriptively and via qualitative thematic analysis to determine key themes and trends.

Results: A total of 17 online survey (n=13) and interview (n=4) responses were collected from February to October, 2022. Most plants were located in Central Ontario (35.3%; n=6). More than 50% (n=9) of respondents were owners of their plant, while the others identified their role as 'manager' (17.6%; n=3), 'director' (11.8%; n=2), 'human resources' (5.9%; n=1), 'butcher' (5.9%; n=1), or 'sales' (5.9%; n=1). Most respondents (47.1%; n=8) indicated that they have worked at their plant for 11-20 years. Thematic analysis yielded five themes: important food safety practices, challenges, solutions and opportunities, impact of COVID-19, and food safety trainings. The participants stated that sanitation, temperature control, training, pre-operational inspections and food safety culture are the most important parts of engaging in food safety practices. While the meat industry in Ontario faces significant challenges (e.g., capital and high turnover rate), smaller and large processors have shared various solutions and opportunities (e.g., high quality and safety standards for products, strong food safety culture, effective communication, and employment opportunities) related to implanting food safety practices and GMPs.

Significance: This study has identified critical factors for successful implementation of food safety practices and GMPs, which can help to promote and encourage effective food safety behavior change in the RTE meat sector.

P2-66 Inhibitory Effect of Organic Herbs on *Listeria* and *Salmonella* Growth: The Influence of Growth Medium

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Introduction: Salmonellosis and listeriosis are foodborne illnesses in humans that case mild to serious symptoms. A wealth of information is available regarding the growth of these pathogens, whereas the inhibitory effect of various agents (essential oils, preservatives etc.) and technologies (use of probiotics, high hydrostatic pressure) on their growth was monitored in several studies.

Purpose: This study aimed to evaluate the effect of organic herbs on the growth of *Salmonella* and *Listeria* in a cooked chicken broth (B) and a laboratory meat broth (ME).

Methods: Approximately 100 cells of *Salmonella* Enteritidis and *Listeria monocytogenes* were inoculated in meat broths containing organic oregano, thymus, summer savory and crithmum as well as mixtures of them. The effect of these herbs, on pathogens' growth parameters was monitored by microplate reader for 24 hours at 37°C. Two growth media were used including the laboratory medium meat extract (ME) broth and chicken meat broth (B) which was prepared by cooked meat to better simulate the food matrix.

Results: According to the obtained results the organic herbs influenced the growth of the pathogens. The observed differences were related to the herb species, the added quantity and the growth medium used. The observed differences between the effect of the same herb on the growth of *Salmonella* and *Listeria* in different growth media, highlight the importance of choosing the most appropriate medium to simulate the food matrices.

Significance: This observation could be fundamental for understanding the challenges that may be faced by transferring the laboratory knowledge to industrial environment and the possible actions to be taken for controlling the pathogens on food, food chain and food processing environment.

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P2-67 Occurrence and Phenotypic Resistance of *Salmonella enterica* from Meats and Related Sources in One Health Concept

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Introduction: The occurrence of antibiotic-resistant foodborne pathogens in meats, meat products and their related samples is a concern worldwide, and a one health approach to tackling this phenomenon is warranted.

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Purpose: This study determined the presence and antibiotic resistance of *Salmonella enterica* from meats and it related sources using one health concept.

Methods: The isolation of *Salmonella* species was done following the procedure in the US FDA, BAM (Andrews et al., 2018). Ten (10) each of raw and RTE beef, chevon, guinea fowl, mutton, pork and chicken, and hand, knife, table and utensil swabs of fresh and RTE meat sellers were sampled. The antimicrobial resistance test was done using the disk diffusion method (Bauer et al., 1996).

Results: The overall occurrence of *Salmonella enterica* in the meat and it related sources was 22.5% (45/200). *Salmonella enterica* were highest in knife swabs from raw meat sellers (70.0%), followed by knife swabs from RTE meat sellers (50%) and raw beef samples (50%). *Salmonella enterica* were not detected in raw chevon, utensil swabs from raw meat sellers and table swabs from RTE meat sellers. Multidrug resistance (resistance to ≥3 different classes of antibiotics) was found in 35% (16) of the isolates. The percentage resistance to one and two antibiotics were 28.9% and 33.3%, respectively. Resistance to tetracycline (37.8%) and amoxicillin (35.6%) was observed. Intermediate resistance was 26.7% for ceftriaxone and 20% for gentamicin. One *Salmonella enterica* isolated from RTE chicken was resistant to as many as eight (amoxicillin-azithromycin-ceftriaxone-ciprofloxacin-teicoplanin-trimethoprim-tetracycline-imipenem) different antibiotics. Susceptibility was high for imipenem (95.6%), chloramphenicol (84.4%), trimethoprim (82.2%), ciprofloxacin (77.8%) and gentamicin (75.6%).

Significance: Some raw meats and RTE meats are contaminated with antibiotic-resistant *Salmonella enterica,* posing risk for their consumption. Proper cooking or reheating is warranted before consumption.

P2-68 Cross-Contamination and Transfer Rates of *Salmonella enterica* Attached and Embedded in Biofilms Formed on Plastic Surfaces to Cooked Chicken

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Introduction: *Salmonella enterica* can form biofilms on inert surfaces used during food preparation which could lead to cross-contamination scenarios.

Purpose: To simulate cross-contamination scenarios and determine transfer rates (TR) of *S. enterica* cells attached or embedded in biofilm formed on plastic surfaces to cooked chicken.

Methods: Three *S. enterica* strains were used (S1, S2 and S3). Plastic surfaces (2x2 cm) were immersed in chicken extract (10 % w/v) inoculated with native microbiota and each *S. enterica* strain (~ 4 log CFU/mL) and incubated at 25°C/4 h). After 4h incubation, surfaces were rinsed to remove non-adhered cells. Some surfaces were used with only attached cells, and others were incubated to promote biofilm formation. These surfaces were incubated for three days at 25°C with 97% relative humidity. The cross-contamination scenarios were simulated by cutting cooked chicken fillet (2x2 cm) on the surfaces containing attached cells or cells-embedded biofilms. *S. enterica* populations was quantified on surfaces and in chicken fillets before and after cutting. The TR was calculated in the different scenarios using the following equation: [CFU_recipient/(CFU_donor + CFU_recipient)]*100. ANOVA and Tukey test were performed for statistical analysis.

Results: Higher TR was observed when *S. enterica* strains were only attached to the plastic surfaces (25.8 to 33.5%) rather than inside a biofilm (2.5 to 4.7 %). Higher TR were observed in strains S1 and S3 when the cells were only attached, and in S2 when the cells were inside the biofilm ($p<0.05$). The *S. enterica* population inside the biofilm increased from 1.1 to 1.5 CFU/surface. The concentration of *S. enterica* in cooked chicken when simulating cross-contamination scenarios was similar when using plastic surfaces containing attached cells (3.4 to 4.1 UFC/chicken fillet), to those surfaces with cells embedded in biofilms (3.6 to 4.1 CFU/chicken fillet).

Significance: Transferring of attached *S. enterica* cells to plastic surfaces occurred easily favoring cross contamination.

P2-69 Effect of Cooling Rates (2-Step vs. Continuous) on the Germination and Outgrowth from *Clostridium perfringens* Spores in Roast Beef, Ham, and Turkey Product

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❖ ***Developing Scientist Entrant***

Introduction: Rapid cooling of meat and poultry products subsequent to cooking (stabilization) is necessary to prevent germination and outgrowth of spores of *Clostridium botulinum*, *C. perfringens*, and *Bacillus cereus*.

Purpose: Evaluate the germination and outgrowth of *C. perfringens* spores following USDA-FSIS Stabilization Guidelines [6.5 h two-step (Rate 1), 6.0 h two-step (Rate 2), and a 6.5 h continuous cooling (Rate 3)] in roast beef, ham, and turkey.

Methods: Raw meat was ground separately and mixed with a marinate containing water, salt, and phosphate (10%, 1.0%, and 0.3% of the finished product, respectively). The meat was portioned (5 g) into a vacuum bag and inoculated with three-strain *C. perfringens* spore cocktail to achieve ca. 2.5 log CFU/g. The bags were vacuum sealed, heat shocked for 20 min at 75 °C , and chilled from 54.4 °C (for cooling rates 1, and 3) or 48.9 °C (cooling rate 2) to 4.4°C within the specified times. *C. perfringens* populations were enumerated on Tryptose Sulfite Cycloserine agar. Data were analyzed using a two-way ANOVA model with PROC MIXED in using $\alpha=0.05$.

Results: *C. perfringens* spore germination and outgrowth for roast beef (0.52, 0.46, and 0.37 log CFU/g) were observed subsequent to cooling, following cooling rates 1, 2, and 3, respectively. Similarly, *C. perfringens* spore germination, and outgrowth by <1-log CFU/g were observed for ham (0.23, 0.48, and 0.33 log CFU/g) and turkey breast (0.16, 0.09, and 0.31 log CFU/g) following the cooling rates 1, 2 and 3, respectively. All three cooling rates meet the USDA-FSIS Stabilization Performance Standard for *C. perfringens* spore germination and outgrowth.

Significance: The two-step cooling of meat and poultry products with rapid cooling from either 54.4 or 48.9 °C to 26.7 °C , followed by slower cooling to 4.4 °C is not necessary to meet the USDA FSIS Stabilization Performance Standards.

P2-70 Correlating *Clostridium botulinum* Growth with Botulinum Neurotoxin Production Using the Dig-ELISA in Model Meat Systems

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✦ ***Undergraduate Student Award Entrant***

Introduction: Our laboratory identified growth characteristics for *Clostridium botulinum* in cooked, uncured beef, chicken and pork at isothermal conditions. However, there remains no consensus on the level of *C. botulinum* growth required for detectable botulinum neurotoxin (BoNT) production in foods.

Purpose: To correlate *C. botulinum* log growth with time to toxicity in model chicken, pork, and beef meat systems stored at temperatures ranging from 25-43˚C using digoxigenin (DIG) enzyme-linked immunosorbent assay (ELISA).

Methods: Ground chicken, pork, and beef were inoculated with a proteolytic cocktail (5 type A and 5 type B) of *C. botulinum* spores (2 log spores/g). Samples were vacuum-packaged, cooked to 71°C, chilled in ice-water, and incubated isothermally at temperatures ranging from 25-43°C. At specified time intervals triplicate samples were removed and *C. botulinum* spores were enumerated on Differential Reinforced Clostridial Agar (dRCA). BoNT was detected using DIG-ELISA and results were verified using the mouse bioassay. Each experiment was conducted twice.

Results: The earliest time to toxicity was consistent for all three meat types stored at 30, 35, 37 and 43°C with toxin detected at 3 weeks, 8 days, 24, 18, 18 and 18 hours, respectively. Slight variability was observed at the other temperatures, with toxin detected at 48-, 48, and 36-h at 25°C, and 18-, 18-, and

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12-hours at 40°C for chicken, pork and beef, respectively. The minimum log change in populations that correlated to Type A toxin detection was 2.96 log at 30°C (chicken), 2.4 log at 25°C (pork) and 2.05 log at 30°C (beef).

Significance: The log growth (2.05-2.96 log increase) associated with toxin production in uncured meats detected by DIG-ELISA is consistently greater than the 0.3-log increase reported by USDA and is consistent with other studies that suggest at least a 2.0-log increase is needed for detectable toxin.

P2-71 Inhibition of *Clostridium perfringens* and *Bacillus cereus* by Commercial Dry Vinegar or Cultured Sugar-Vinegar Blends during Extended Cooling of Model Uncured Beef and Poultry Products

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Introduction: The 2021 FSIS Stabilization Guidelines for Meat and Poultry Products limits Phase1 cooling from 48.8 to 27°C in uncured meats to 1 hour. However, this time restriction is impractical for use in large diameter whole muscle products.

Purpose: To compare the inhibitory effect of dry vinegars (DV) and cultured sugar-vinegar blends (CSV) on *Clostridium perfringens* and *Bacillus cereus* in uncured beef and poultry products during extended cooling.

Methods: Treatments (beef: 72-73% moisture, pH 6.2-6.3, 0.85-0.95% NaCl; turkey: 76-77% moisture, pH 6.5-6.7, 1.3-1.6% NaCl) included control without antimicrobials, plus four DV and four CSV tested at 0.75 and 1.25%. Sub-batches were inoculated with 2.5 log *C. perfringens* or *B. cereus* spores, vacuum-packaged, and cooked to 73°C. Packages were cooled from 48.8 to 27°C (Phase1) in 3, 4, and 5 hours; Phase2 (27 to 12.8°C) and Phase3 (12.8 to 4°C) were standardized for 5 h cooling each. Pathogens were enumerated on selective agar in triplicate samples assayed at pre-cook, post-cook, and at the end of phase 1, 2, and 3 cooling. Each set of experiments were conducted twice.

Results: B. cereus did not grow in any treatment when Phase1 cooling was extended to five hours. As expected, *C. perfringens* grew rapidly (2.5 to >4.5 log) in Control treatments when Phase1 cooling was extended to ≥3 h. 1.25% DV (all suppliers) limited growth to ≤1 log when Phase1 cooling was extended to 3 h but supported >1 log increase when Phase1 cooling was extended to 5 h. 1.25% CSV (all suppliers) inhibited *C. perfringens* growth under 3 h Phase1 cooling; 1.25% CSV-A and ≥0.75% CSV-D inhibited growth in Turkey during 5 h Phase 1 cooling, but inhibition was inconsistent in beef.

Significance: Formulating uncured meats with 1.25% DV or certain CSV can extend Phase1 cooling to 3 hours. Greater variability of inhibition was observed among CSV than for DV, although all ingredients inhibited growth when used at ≥0.75% compared to a control.

P2-72 Effect of Dry Vinegar Flavor on Three Important Foodborne Pathogens and Shelf Life of Raw Ground Beef during Cold Storage

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❖ ***Developing Scientist Entrant***

Introduction: Bacteria present on the surface of beef carcasses and cuts can be integrated into the beef during grinding and multiply rapidly, causing foodborne outbreaks and shortening shelf life. Antimicrobial interventions applied to ground beef can reduce spread of foodborne pathogens.

Purpose: To evaluate the efficacy of dry vinegar in ground beef on foodborne pathogens and shelf life during refrigeration.

Methods: Triplicates of 6 raw ground beef samples were inoculated individually with *E. coli* O157, *Salmonella sp*., or *L. monocytogenes* (ca. 4 Log CFU/g) and incorporated with 0.5% and 0.8% dry vinegar flavor (Galimax V-100 (Galactic Inc, WI, USA)), respectively. Samples were stored under refrigeration, and bacterial enumeration was conducted after 0, 4, 8, 12, and 24 hours, 2, 4, and 6 days. The effect of the dry vinegar treatments on ground beef shelf life was studied by enumerating *E. coli*/coliforms and psychrotrophic bacteria on uninoculated ground beef samples stored under refrigeration. Bacteria were enumerated after 0, and 8 h, 1, 3, 5, 7, and 14 days. Bacteria were enumerated using conventional plating techniques and colony counts were log-transformed. Repeated measure Analysis of Variance (ANOVA) was applied to test the effect of the treatment and correlation in the bacteria counts over time.

Results: There was a time-dependent effect of dry vinegar treatment on microbial concentration of all bacteria tested (P<0.01). The average counts of *Escherichia coli* O157, *Salmonella*, and *Listeria monocytogenes* in ground beef were consistently lower in both treatment groups than the control group (P<0.01). For *E. coli*/coliform and psychrotrophic bacteria, the average bacterial count did not show an increase for both vinegar-treated groups compared with control groups (P<0.01).

Significance: Adding dry vinegar flavor to ground beef suppresses important pathogenic bacteria, as both treatments showed a bacteriostatic effect. Treatments improved shelf life during refrigeration for up to 14 days.

P2-73 Long Come-up-Time HACCP Deviation and *Staphylococcus aureus* growth during Cooking of Beef Products

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

Purpose: This study evaluated the growth of *Staphylococcus aureus* in beef formulations during cooking from 50 to 130°F in 9.3-hours to represent a HACCP deviation case.

Methods: Irradiated ground beef formulations- meat without antimicrobial (C), meat with liquid-vinegar @ 2% (V), or lactate-diacetate @ 2.5% (L), were evaluated. An individual experimental unit consisting of a 5-g portion of meat in a plastic bag, inoculated with a four-strain cocktail of *S. aureus*, was spread thin in the bag. 50 units per formulation were heated together in a water bath to a time and temperature profile similar to a HACCP deviation case in a commercial establishment. *S. aureus* populations 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. Populations were transformed to log scale and reported as mean±SD of the trials.

Results: Formulations had pH, moisture, and salt content of 6.3-6.5, 74%, and 0.6%, respectively. The hourly temperatures of meat from 0 to 9 hours were 50, 66, 80, 89, 99, 106, 113, 120, 124, and 128°F, and reached 130°F at 9.3 hours. *S. aureus* count at time 0 was 3.0±0.1 log CFU/g. Maximum growth of 1.1±0.2, 0.2±0.1, and 0.9±0.3 log was observed in the sixth hour in formulations C, V, and L, respectively (P<0.05). The counts decline thereafter (P<0.05). In comparison, available validated models, UW Therm and DMRI Staphtox Predictor, after adjusting the temperature limitations per Appendix-A guideline, estimated 4.2 and 3.3 log increase, respectively, over-conservatively suggesting unsafe meat products.

Significance: Challenge study may help processors validate long come-up-time.

P2-74 Comparison of Sous Vide Cooking Parameters for *Salmonella enterica* and *Listeria monocytogenes* Inactivation in Intact and Blade-Tenderized Beef Steaks

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Introduction: Sous-vide, cooking of vacuum-packaged products in water at relatively low temperatures and longer time, is popular in food service and home kitchens. Our previous study assessed time-temperature combinations for cooking serving-size intact steaks. Parameters for blade-tenderized steaks should be determined as inactivation of internalized pathogens may require a longer time.

Poster

Purpose: This study aimed to evaluate time-temperature combinations for cooking intact and blade-tenderized steaks and determine if cooking time is affected by location of pathogens in steaks.

Methods: Steaks (100g, n=182) were surface inoculated with ~8 logs of five strains of *Salmonella enterica* and three strains of *Listeria monocytogenes*. Non-intact steaks were blade tenderized using a jaccard. Steaks were individually vacuum packed and cooked at 52.5°C , 57.5°C , and 60°C for up to 450 min, 90 min, and 54 min, respectively, with eleven, seven, or eight incremental samples. Studies were independently replicated three times. Data were analyzed using mixed model with steak type, cooking temperature, and time as fixed effects and replicates as random effects.

Results: A 5-log *Salmonella* reduction in non-intact steaks required more time (P<0.027) than intact steaks. In non-intact steaks, the reduction was achieved after 135 mins and 45 mins at 52.5°C and 57.5°C , respectively, while in intact steaks, the reduction required 90 mins and 30 mins. However, at 60°C , a 5-log reduction was achieved in 24 mins and not influenced (P=0.309) by blade tenderization. For *Listeria monocytogenes*, 5-log reduction at 52.5°C and 57.5°C was achieved at 315 min and 60 min, independent of blade tenderization (P>0.492). In contrast, at 60°C , a 5-log reduction was achieved after 32 min for blade tenderized and 40 min for intact steaks (P=0.0008).

Significance: The data suggest that the cooking time can be adjusted based on the steak type to achieve desired eating characteristics while mitigating food-safety risks.

P2-75 *Sous-Vide* Safety: Evaluating *Sous-Vide* Cooking Parameters of Contaminated Beef Products

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Introduction: *Sous-vide* cooking is a growing trend among retailers and consumers. Foodborne pathogens may survive the cooking if non-validated parameters are used or if pathogens have enhanced thermal resistance.

Purpose: Pathogen inactivation from *sous-vide* cooking was determined when introduced directly to beef products or via contaminated spices, and with or without a finishing step.

Methods: Beef products (ground beef, tenderized steaks, non-tenderized steaks) were inoculated with pathogens (*Salmonella* Montevideo and *Escherichia coli* O157:NM) three ways: 1) directly onto the meat, 2) via inoculated ground black pepper incorporated into the recipe, and 3) via inoculated ground pepper equilibrated at 30% RH for four days prior to incorporation. Samples were vacuum-packaged and submerged in a water bath for 120 mins at 62.5°C. Samples were sampled at predetermined time intervals. A duplicate sample was grilled to a specific internal temperature (74°C for ground beef, 57 °C for steaks) and sampled. Pathogen reductions were analyzed with ANOVA and pairwise comparisons.

Results: *Sous-vide* cooking lowers pathogens by more than 5 log CFU/g in most cases, but to a significantly lower extent for all products than that of grilling (p<0.05). There were not statistically significant differences between inoculation methods. Tenderization of steaks resulted in significantly lower reductions of pathogens from *sous-vide* cooking (p<0.05). STEC reductions were consistently lower than those of *Salmonella*, regardless of inoculation and cooking method or time, though not significantly in most cases.

Significance: This research validated *sous-vide* cooking parameters (120 mins, 62.5°C) and found that *sous-vide* cooking alone reduced pathogen populations, grilling was required for a 5-log reduction in some instances. The introduction of pathogens via contaminated pepper led to less consistent reductions during the cooking process, but results indicate that 2-hr *sous-vide* cooking is still sufficient for a 5-log reduction. Cooking instructions must be validated as *sous-vide* products are marketed more to restaurants and consumers.

P2-76 Validation of the GENE-UP® *Salmonella* and EHEC Methods for the Detection of *Salmonella* spp. and Enterohemorrhagic *Escherichia coli* in Sampling Cloth from Beef

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Introduction: The GENE-UP® SLM (*Performance Tested Method*℠ 121802) and EHEC (*Official Method of Analysis 2020.06*) assays are real-time PCR molecular detection methods that utilize Fluorescence Resonance Energy Transfer proprietary hybridization probes for the rapid detection of *Salmonella* spp. and Enterohemorrhagic *E. coli* (EHEC) in food and environmental samples.

Purpose: To conduct AOAC matrix extension studies of the candidate methods for the detection of *Salmonella* spp. and STECs in MicroTally cloth from beef trim.

Methods: The candidate methods were evaluated according to the requirements of AOAC Appendix J validation guidelines. The validation included the evaluation of one matrix (MicroTally cloth from beef trim) at three contamination levels: 20 replicates at a low-level of 0.2–2.0 CFU/test portion, five replicates at a high-level of 2–10 CFU/test portion, and five replicates at a non-inoculated control level of 0 CFU/test portion. Test portions were evaluated using a direct lysis procedure and an immunomagnetic concentration procedure. Test portions were confirmed following procedures in the USDA FSIS MLG. For all methods, two enrichment media were evaluated, buffered peptone water (BPW) and modified tryptic soy broth (mTSB). The following EHEC series assays were evaluated, Pathogenic E. coli (PEC), stx/eae (EH1), *Escherichia coli* O157:H7 (ECO) and Top 6 STECs (EH2).

Results: The results of the study indicated no differences between presumptive and confirmed results for both candidate methods using either enrichment broth. When compared to the reference method for STECs, the candidate method demonstrated higher recovery of the target analyte. For *Salmonella*, there were no statistically significant differences observed between the candidate method and the USDA MLG method.

Significance: The GENE-UP SLM and EHEC series can be utilized in place of traditional MLG procedures to provide rapid detection of *Salmonella* spp. and STECs from cloth sampling test portions incubated in as little as 8 hours.

P2-77 An Evaluation of the GENE-UP® QUANT Salmonella Method for the Detection and Enumeration of *Salmonella* spp Contamination as Low as 1 CFU per gram in Ready-to-cook or Ready-to-heat Products

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Introduction: Salmonella is estimated to cause approximately 64% of the pathogenic bacterial foodborne outbreaks occurring per year in the United States. Until recently, FSIS quantification was performed using a most probable number (MPN) methodology taking from 4-7 days. The evaluated method includes a real-time multiplex PCR assay for the rapid detection and enumeration of Salmonella species targeting highly-conserved pan-Salmonella genes while accommodating both an enrichment-free and an abbreviated enrichment-based sample prep.

Purpose: To evaluate the performance of the candidate method according to AOAC® PTM℠ Guidelines versus USDA FSIS/MLG Appendix 2.05

Methods: Twenty unpaired 325g replicates of pre-screened ready-to-heat chicken cordon bleu were enriched with 975mL of prewarmed BPW and homogenized 30s before incubation at 35 ± 2°C for 4 ± 0.25h. Samples were contaminated with *Salmonella typhimurium* at four levels: N=5 at 0 CFU/g (uninoculated), N=5 at 1-10 CFU/g (low), N=5 at 10–100 CFU/g (medium) and N=5 at 100–1,000 CFU/g (high). Assay test portions were prepared through DNA concentration, extraction and purification of 40ml post-incubation aliquots. Prepared test portions were analyzed via PCR, quantified via proprietary external algorithm and confirmed according to MLG 4.10.

Results: Logarithmic transformation of the CFU/g for each contamination level was performed at the 95% confidence interval and the difference between the means was determined at the both the 90 and 95% intervals. Best estimate of the true difference between methods was calculated as 0.31 for low, 0.26 for medium and 0.20 for the high level. For each level, the mean difference between methods was < 0.32 log indicating comparable results. Repeatability of the candidate method was found to be lower or identical to the reference method, indicating a high level of precision.

Significance: The sensitivity and repeatability of the GENE-UP® QUANT Salmonella method allows dependable, accurate results in as little as 6.5 hours for contamination as low as 1 CFU/g.

P2-78 *Salmonella* Quantification (SalQuant®) with the Hygiena® BAX® System for Beef Carcass Swabs

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Introduction: The microbiological hygiene of cattle at slaughter is an important requirement to properly assess contamination risks for meat entering the ground beef supply. Research studies have found a strong correlation between hide and carcass contamination with *Salmonella* and the levels of *E. coli* O157:H7 present. Typically, these pathogens are only tested for prevalence which does not allow the resultant transfer rates to be well understood.

Purpose: The use of quantitative methods can provide more valuable data and insight on managing pathogens in targeted reduction efforts. The objective of this study was to develop a linear equation and verify the capability of a real-time PCR assay for *Salmonella* quantification (SalQuant®) in beef carcass swabs.

Methods: Beef carcass swabs provided by an industry partner were artificially inoculated with *Salmonella* at four levels (10, 100, 1,000, and 10,000 CFU/ml) for quantification. Following inoculation, swabs were enriched in 50 ml of pre-warmed (42 °C) MP media, incubated and then tested in quintuplicate using real-time PCR. At the same time, a 3-tube x 5-dilution MPN for each inoculation level was prepared following the USDA FSIS Appendix 2.05. Data was assessed using linear regression to compare the cycle threshold values (Ct) and known inoculation levels to create a best-fit equation.

Results: The eight-hour enrichment produced the best linear fit equation with a R^2 of 0.87 and log_{10} RMSE of 0.43. When comparing estimations from the model to MPN results, there were no statistical differences.

Significance: These results demonstrate accurate and rapid quantification of *Salmonella* from 1.0 to 4.0 log CFU/mL from beef carcass swabs can be achieved using the BAX® System Real-Time S*almonella* PCR assay.

P2-79 *Salmonella* Quantification (SalQuant®) with the Hygiena® BAX® System for Breaded Stuffed Raw Chicken Products

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Introduction: On August 1, 2022, the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA FSIS) announced new regulatory action to declare *Salmonella* an adulterant in breaded, stuffed, raw chicken products.

Purpose: To meet the proposed USDA testing requirements for these product types, an AOAC *PTM-certified* real-time PCR method was used to develop a quantification curve for *Salmonella*.

Methods: Four varieties of breaded, stuffed, raw chicken products provided by an industry partner were prepared separately in four individual studies. Samples (375 g) were divided into 15 test portions per matrix and inoculated with a cold-stressed culture of *Salmonella* across 5 target concentrations. One additional sample was left for a negative control. Samples were homogenized with equal parts of BPW and then 30 mL was transferred into a new container. Samples were enriched with pre-warmed (42 °C) MP media with Quant™ Solution. After a short incubation time, samples were tested by real-time PCR. Regression analysis was used to find the best-fit line for each matrix, and then the combined mean was used to generate one equation. Final estimations were then compared to MPN results.

Results: Eighty (80) data points were generated for each matrix. The mean from all 4 matrices (320 data points total) produced a combined equation with an R^2 of 0.86 and Log RMSE was 0.57 at 6 hours. There were no statistical differences between MPN values and SalQuant estimations at any inoculation level.

Significance: The results demonstrate rapid and accurate quantification of *Salmonella* across a range of 1 – 10,000 CFU/g using the BAX® System SalQuant® method.

P2-80 Matrix Validation of 375 Ml Spent Sprout Irrigation Water for the Detection of *E. coli* O157:H7 and *Salmonella* Using the Hygiena® BAX® System

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Introduction: Pathogen contamination of sprouts has been a recurring public health challenge. Three critical control points of sprout production have been identified: the seeds, spent irrigation water and the sprouts (finished product). Growers can use approved seed treatments to reduce pathogens prior to sprouting, but this does not guarantee complete pathogen elimination. Therefore, it is equally important to perform microbiological testing of the spent irrigation water.

Purpose: This study was designed to validate spent sprout irrigation water for the detection of *E. coli* O157:H7 and *Salmonella* using a rapid, real-time, PCR-based method.

Methods: An unpaired matrix study was performed following the technical guidelines in AOAC INTERNATIONAL Official Methods of Analysis Appendix J. Briefly, spent sprout irrigation water was co-inoculated with *E. coli* O157:H7 and *Salmonella* Enteritidis at ≤ 1 CFU/test portion to create a low fractional level and at 5 to 10 CFU/test portion to create a high level. Samples were equilibrated at 4°C for approximately 72 hours. Test method samples (375 ml) were enriched in BPW and tested by real-time PCR, while reference method samples (100 ml for *E. coli* O157:H7 and 375 ml for *Salmonella*) were enriched and confirmed according to their respective procedures in the FDA guidance documents.

Results: Test method samples returned fractional positive results for *E. coli* O157:H7 in 8/20 and for *Salmonella* in 5/20 low-level inoculated test portions, while all high-level inoculated test portions were positive. All real-time PCR results were confirmed by culture. When compared to the reference method using the probability of detection (POD), there was no significant difference for either target organism.

Significance: The BAX® System Real-Time PCR assays for *E. coli* O157:H7 Exact and for *Salmonella* both demonstrated a sensitivity rate of 100% and a specificity rate of 100% for the detection of *E. coli* O157:H7 and *Salmonella* with statistical equivalence to the FDA reference methods.

Poster

P2-81 Inactivation of *Salmonella* Enteritidis in Raw Eggs Using Bacteriophage Cocktails

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* Enteritidis is currently the most common bacterial pathogen associated with eggs. Current interventions adversely affect egg composition and primarily decontaminates the egg surface. Bacteriophages (phages) have been suggested as a novel antimicrobial due to their high specificity and safety advantages.

Purpose: This study was conducted to investigate the antimicrobial effect of phage cocktails for reducing *Salmonella* contamination in raw egg yolk and egg white.

Methods: Raw egg white and egg yolk were inoculated with 5 log CFU/ml of three *Salmonella* Enteritidis strains and treated with four phage cocktails to yield multiplicities of infection (MOI) of 100 and 1000, the samples were then incubated at 8°C for up to 30 days. *Salmonella*-inoculated egg samples without phage treatment served as the control. The population of *Salmonella* cells were enumerated at 0 h, 6 h, day 1, 6, 12, 20 and Day 30 on tryptic soy agar (TSA). Each experiment was independently conducted three times.

Results: The population of all three strains of *Salmonella* Enteritidis were reduced significantly in both egg white and egg yolk treated with phages. While three of the four phage cocktails with MOI of 1,000 reduced the population of strain S47 by > 3 log (P<0.05) in egg yolk, the remaining phage cocktail reduce the population of strain S35 and S26 by 1 to 2 log (P<0.05) on day 30. In egg white, three phage cocktails with MOI of 1,000 resulted in a complete inactivation (P<0.05) for strains S35 and S47 after 12 days, whereas only one phage cocktail reduced strain S26 population by 4 log on day 30 (P<0.05).

Significance: This study demonstrated that phage application to both egg yolk and egg white can reduce *Salmonella* contamination at low incubation temperature, without regrowth during long-term storage, but the magnitude of the reduction was both strain- and phage-dependent.

P2-82 Development of Stable High Internal Phase Pickering Emulsions Constructed from Egg Yolk Low Density Lipoprotein-Pectin Complexes as Potential Fat Substitutes

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❖ ***Developing Scientist Entrant***

Introduction: High internal phase Pickering emulsions (HIPEs) is considered as the best candidates for fat substitution due to their high stability, environmental friendliness and sustainability.

Purpose: To evaluate the feasibility of using egg yolk low density lipoprotein (LDL) and egg yolk low density lipoprotein-pectin blends (LDL-PE) as natural nanoparticle stabilizers for the preparation of HIPEs.

Methods: LDL was extracted by salting precipitation, and the extracted LDL was diluted to 20 mg/mL using ultrapure water, and pectin (PE) to 20 mg/mL. To investigate the effect of pH, the two solutions were blended at a mass ratio of 1:0.5 and the pH of LDL-PE dispersions was adjusted to 3, 4, 5, 6 and 7. To explore the effect of mass ratio, the LDL was fixed at 10 mg/mL, and the mass ratios of LDL and PE were adjusted to 1:0.17, 1:0.33, 1:0.5, 1:0.67 and 1:1. Finally, nanoparticles at concentrations of 0.5% to 2.0% were added to soybean oil to obtain HIPEs with an internal phase volume fractions of 70% to 90%.

Results: The average particle size of LDL was 49 nm and the contact angle was nearly 90°. LDL-PE binds via electrostatic interactions. The HIPEs prepared from 2% LDL and 80% oil were the best formulation at neutral pH or higher PE concentration after centrifugation and 4 weeks of refrigeration at 4 °C or heating at 90 °C. Finally, superior photostability against UV radiation was obtained by encapsulating curcumin in HIPEs with 80% retention and elevated bioaccessibility from 10% to 50% during in vitro digestion.

Significance: HIPEs prepared from natural ingredients have the potential as trans-fat substitutes and delivery vehicles.

P2-83 Ceca May Not Serve as an Adequate Predictive Sample for *Salmonella enterica* in Ground Turkey

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Introduction: Although ceca have been considered a "gold standard" sample for *Salmonella* detection in poultry, there is evidence that they are inadequate for predicting ground turkey test results.

Purpose: This study evaluated the effectiveness of ceca as a predictive sample of *Salmonella* in ground turkey.

Methods: *Salmonella* quantification was conducted utilizing the Hygiena SalQuant™ Real-Time PCR assay, following manufacturer recommendations. Briefly, homogenates were made by adding one smashed ceca to 100mL of pre-warmed (42°C) BAX MP with antibiotic. Ground turkey homogenates utilized the same enrichment procedure, comprising of 25g sample to 25 mL of media. 1:1 enrichments were made by aliquoting 30mL of the cecal homogenate to 30 mL of pre-warmed (42°C) BAX MP. 1:1 enrichments were incubated at 42±1°C for 10 hrs. Samples that were not quantifiable after 10hrs were incubated until 24hrs and evaluated for presence/absence on the BAX system. Samples that were negative for SalQuant but positive for detection were considered positive but below the limit of quantification. Microbial counts were converted to log10 CFU/g.

Results: A total of 14 turkey barns were sampled. From these barns, 140 ceca were collected, and 60 grind sample were collected. Of the total 140 ceca, four tested positive for *Salmonella* (2.86%), whereas 36 of the 60 grind samples tested positive (60.00%). *Barn 3* was the only barn sampled where both ceca and grind samples tested positive. *Barn 3* was also the only barn in which a ceca sample was quantifiable (1.34 log10 cfu/g). Interestingly, all grind samples collected from *Barn 3* tested positive at quantifiable levels. *Barn 3* also had the highest quantification values (average of 1.24 log10 cfu/g).

Significance: Data indicates that quantification of *Salmonella* within ceca is more predictive than detection. However, overall, this data indicates that ceca samples may not serve as a quality predictive sample for final ground turkey test results.

P2-84 High-Resolution Serotyping to Improve *Salmonella* Surveillance in Turkey

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Developing Scientist Entrant

Introduction: Despite extensive *Salmonella* mitigation strategies in turkey production, 5.9% of salmonellosis cases are linked to turkey. There is a need for effective *Salmonella* screening in turkey production.

Purpose: To determine *Salmonella* prevalence and serovar diversity through live-production and processing stages of turkeys.

Methods: Farm (one bootsock pair, collected directly after harvest), pre-chilled processing (pre-scald, ceca, and pre-chill rinses (n=6 composites of 10 for each sample type)), and final product (mechanically separated turkey (MST), and ground turkey (n=6 bins of each)) samples were each collected from 22 turkey flocks. *Salmonella* prevalence for each sample was determined by PCR and culture. Deep serotyping by CRISPR-SeroSeq was performed on 177 *Salmonella*-positive samples.

Results: At processing, all 22 flocks were *Salmonella* positive at one or more sampling locations and 44.5% (10/22) of farm samples were positive. *Salmonella* prevalence decreased from pre-scald (56.1%, 74/132) to pre-chill (18.2%, 24/132)(Mann-Whitney U test, $p<0.05$), demonstrating the efficacy of antimicrobial wash steps. Prevalence increased from pre-chill to MST (66.2%, 88/133; $p<0.05$) and pre-chill to ground turkey (50.4%, 67/132; $p<0.05$), suggesting release of internalized *Salmonella* during further-processing. In 84.2% of flocks (16/19), deep serotyping detected at least one serovar in MST or ground turkey that was absent upstream. Overall, 57 of 177 *Salmonella* positive samples (32.2%) contained two or more serovars including 27 pre-scald samples (48.2%). All cecal and pre-chill samples contained a single serovar. In total, 18 serovars were detected; Typhimurium (26.1%), Hadar (21.7%), and Uganda (16.4%) were most frequent.

Significance: This study demonstrates the ineffectiveness of farm sampling at harvest to indicate *Salmonella* prevalence and serovar identity in turkey products, highlighting the challenge for pre-harvest vaccine development for *Salmonella* control. The data also demonstrates presence of different serovars in different final products originating from the same flock, underscoring the complexity of tracking serovars in turkey production.

P2-85 Application of Spectroscopic Technologies for Rapid Quality Assessment of Chicken Breast Fillets

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Introduction: Spectroscopic sensors have been largely investigated as non-destructive, rapid alternatives that could complement the traditional microbiological testing of the end-product through their integration across the food supply chain.

Purpose: To evaluate the feasibility of Fourier transform infrared (FTIR) and fluorescence (Freshdetect portable fluorescence device) spectroscopy in tandem with machine learning algorithms and multivariate data analysis to quantitatively assess chicken meat microbiological quality.

Methods: Chicken breast fillets were obtained in plastic packages from a local industry and stored aerobically at 4°C. Throughout storage, microbiological analysis (n=6) was performed for the enumeration of total viable counts (TVC), *Pseudomonas* spp., *Brochothrix (B.) thermosphacta*, lactic acid bacteria (LAB) and Enterobacteriaceae, while concomitant FTIR and Freshdetect analysis (n=12) was conducted. Spectral data analysis and correlation with microbial counts was performed with two machine learning approaches, namely partial least squares regression (PLSR) and support vector machines regression with radial basis function kernel (SVMR). The developed PLSR and SVMR models were externally validated through independent batch and intra-batch testing, respectively.

Results: The SVMR models based on spectral data from FTIR exhibited generally better performance than the respective PLSR models for the estimation of the different microbial groups. Moreover, the SVMR models predicted more satisfactorily TVC (R^2 0.879, RMSE 0.607), followed by *B. thermosphacta* (R^2 0.873, RMSE 0.654) and *Pseudomonas* spp. (R^2 0.793, RMSE 0.855), than LAB (R^2 0.686, RMSE 0.649) and *Enterobacteriaceae* (R^2 0.477, RMSE 0.801). Contrarily, the developed models based on data from Freshdetect achieved poor prediction performance, irrespective of the algorithm and microbial group examined.

Significance: Results confirm the considerable potential of the spectroscopic technologies (sensors) coupled with the appropriate machine learning approach for the real-time and accurate monitoring of chicken meat quality. However, this potential appears to be sensor-specific, since FTIR-based models outperformed Freshdetect-based models.

Acknowledgement: The Horizon 2020-funded project "DiTECT" (No. 861915).

P2-86 Survey Sampling for *Salmonella* in Raw, Breaded, Stuffed Chicken Products

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Introduction: FSIS coordinated the testing of not Ready-to-Eat breaded, stuffed chicken products for *Salmonella* and sanitary indicator aerobic organism counts by state public health and agriculture laboratories.

Purpose: While raw, these products are pre-browned and may appear ready-to-eat. Their combination of poultry meat and other components differ from most raw chicken samples (e.g., raw chicken parts, ground, or rinsates), making method selection and sample preparation important decisions.

Methods: State laboratories used current validated methods for detecting *Salmonella* in food samples and based on the FDA BAM or FSIS MLG. Methods differed by sample size with 25g and 325g samples being the most common. All laboratories followed the FSIS recommended sample preparation protocol for ready-to-eat products by analyzing a representative poultry portion in combination with other ingredients.

Results: Out of 487 samples that were tested, 58 samples were positive for *Salmonella*. However, *Salmonella* detection differed noticeably depending on whether laboratories used methods the same as FSIS (36 of 135, 27% *Salmonella* positive) or used methods different from FSIS (22 of 352, 6% *Salmonella* positive). Total aerobic counts were similar among samples tested except for those from two establishments that produce a similar product that is not breaded but also appears ready-to-eat. Isolate analysis using whole genome sequencing identified several serotypes: *Salmonella* Enteritidis (18/58), *Salmonella* Infantis (22/58), *Salmonella* Kentucky (15/58) and *Salmonella* Typhimurium (3/58).

Significance: Participating laboratories using the same *Salmonella* detection and sample preparation as FSIS MLG 4.12 found *Salmonella* in these products nearly 27% of the time. This is similar to the rate of 29% found in further processed chicken parts or comminuted product. Serotypes isolated from stuffed chicken fell within the top 5 serotypes from other FSIS regulated chicken products. Results suggest that using FSIS' ready-to-eat sampling protocol and MLG method to detect *Salmonella* is appropriate for analyzing these not ready-to-eat poultry products.

P2-87 Antibiotic-Free Semi-Quantitative Method for Assurance® GDS for *Salmonella* Heidelberg Enteritidis Typhimurium (HET) Tq Assay

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Introduction: *Salmonella* continues to be a highly monitored pathogen in the food industry. Semi-quantitative detection assays can assist poultry facilities in determining where to focus their cleaning efforts when detecting *Salmonella* positive samples.

Purpose: Evaluate a semi-quantitative assay for poultry facility swab samples and to simplify enrichment by eliminating the use of antibiotics while maintaining assay sensitivity.

Methods: Firstly, 9 different background microflora were isolated from a poultry facility. Pure cultures of representative strains of *Salmonella* Heidelberg (SH), Enteritidis (SE) and Typhimurium (ST) were serially diluted in BPBD to create 5 inoculation levels and mixed with $1.7x10^5$ CFU/mL background microflora. The final inoculum was added to 200 mL of Tetrathionate broth (TT) and incubated at 37°C for 24h. Five replicates of each inoculation level were analyzed, and a standard curve was developed. Further, a total of 44 inclusivity strains were grown in BPW at 37 °C for 24h, diluted to 10^{-4} and a total of 62 non-HET and non-*Salmonella* exclusivity strains were grown in BHI at 37 °C for 24h and analyzed using the Assurance® GDS for *Salmonella* HET Tq assay. Finally, to evaluate the levels of total *Salmonella* present in boot swab samples obtained from poultry facilities, samples were enriched in TT, and TT+20 mg/L or 40 mg/L novobiocin and tested using the 20 and 24h.

Results: The HET assay produced a linear response curve in the presence of background microflora and can be categorized into three different levels of contamination: Low (2-100 CFU/200 mL), Medium (100-1000 CFU/200 mL), and High (>10,000 CFU/200 mL), with 100% specificity. Further, the sensitivity of *Salmonella* detection is 97% using TT as the primary enrichment and testing samples at 24h. *Salmonella* growth was inhibited with higher concentrations of novobiocin

Significance: Semi-quantitative assays provide more information to food producers, enabling them to better consider risks and optimize resources.

P2-88 Rapid Method for the Quantification of *Salmonella* spp. Contamination in Poultry Meat

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Introduction: To reduce the *Salmonella* foodborne illnesses attributed to poultry products, the USDA FSIS released a new regulatory framework for a strategy to control the contamination risk.

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Purpose: This change calls for new tools to enable the poultry industry to improve their risk management.

Methods: Pall GeneDisc method for *Salmonella* spp. certified by AFNOR and/or AOAC, enables fast results delivering a negative result in down to 20 h. To improve this time to result and to address quantification issues with a tailored threshold setting, we validated a new protocol with or without minimal enrichment. Poultry rinsate samples have been tested with low levels of spiked stressed *Salmonella* to determine what are the minimal thresholds we can detect and what is the shortest enrichment time to guarantee 1 CFU/ml.

Results: Results highlighted that *Salmonella* strains had no impact on the enrichment time. Thus, the enrichment time for the detection of *Salmonella* spp. in samples only depended on the initial *Salmonella* spp. contamination level. Thanks to the very high sensitivity of the Pall GeneDisc method, a sample contaminated at 1 CFU/mL was called positive by the GeneDisc method after 2 h of enrichment while a quantification can be done without enrichment to enable the setting of a threshold addressing very low levels of contamination.

Significance: The tests conducted in this study utilizing the Pall GeneDisc System *Campylobacter*/*Salmonella* combination plates demonstrated the ability to detect *Salmonella* spp. with minimal enrichment time and to address low levels of contamination without enrichments.

P2-89 Use of a Mobile Methodology for Bio-Mapping of Microbial Indicators and RT-PCR-Based Pathogen Quantification in Commercial Broiler Processing Facilities in Central America

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Introduction: Poultry bio-mapping helps identify microbial contamination and process optimization opportunities such as: sanitary dressing procedures, equipment adjustments, cross-contamination controls, and chemical intervention applications in commercial poultry processing operations.

Purpose: Create a microbiological baseline of microbial indicators including aerobic plate counts (APC), Enterobacteriaceae (EB), and *Salmonella* spp. and *Campylobacter* spp. quantification and detection in four commercial broiler processing facilities in Central America.

Methods: Buffered peptone water was used to collect poultry rinses (n = 200) from different locations: live receiving (LR); rehanger (R); post-evisceration (PE); post-chill (PC) and wings (W). MicroSnap™ system was used for enumeration of microbial indicators APC and EB, BAX®-System-SalQuant™ was used for quantification and detection of *Salmonella* spp., and BAX®-System-CampyQuant™ for *Campylobacter* spp. Negative samples after enumeration were tested with BAX®-System Salmonella and Campylobacter for prevalence analysis. APC and EB counts were reported as Log CFU/mL, ANOVA and pairwise comparison T-test adjusted Tukey (P < 0.05) was performed. Pathogen counts were reported as Log CFU/sample, Kruskal-Wallis and pairwise comparison Wilcoxon's test adjusted Benjamin & Hochberg method (P < 0.05) was performed.

Results: APC and EB counts at LR were 7.66 and 6.05 Log CFU/mL, respectively. After R, counts were significantly reduced to 5.99 and 4.68 Log CFU/mL, respectively. From PC to W counts increased significantly from 1.91 – 1.03 to 5.42 - 2.80 Log CFU/mL, respectively. *Salmonella* spp. counts were significantly different between stages (P < 0.01); prevalence of *Salmonella* spp. was highest at LR 92.5%, while PC was the lowest 15.4%. *Campylobacter* spp. counts were lower than the other stages at W 1.61 Log CFU/sample; however, Campylobacter spp. prevalence was higher than 62.5% in all stages.

Significance: Bio-mapping helped develop statistical process control parameters for individual process in all broiler processing facilities of this Central American country. National baseline data serves as benchmark for continuous improvement at the facilities evaluated.

P2-90 Quantification of *Campylobacter* spp. in Poultry Carcass and Part Rinses Collected at Different Processing Stages in a Commercial Broiler Facility

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Introduction: Poultry products are a well-known source of *Campylobacter* spp., poultry bio-mapping based on pathogen quantification is important to identify contamination levels and implement sanitation and interventions during broiler processing to reduce the risk foodborne illness to consumers.

Purpose: To develop poultry bio-mapping of *Campylobacter* spp. levels in two different processing lines using two different commercial methodologies for pathogen enumeration.

Methods: Whole chicken carcasses (n = 180) from: post-pick (PP); rehanger (R); post-evisceration (PE); post-chill (PC) locations; and 4-pound cut-up wings samples (W) were collected and rinses with 400 mL of buffer peptone water. The Most-Probable-Number-based TEMPO™ system was used for the enumeration of *Campylobacter* spp. In addition, BAX®-System-CampyQuant™ was used for the quantification and detection of *Campylobacter* spp. BAX®-System-*Campylobacter* prevalence analysis was performed only for negative enrichments ran for quantification. *Campylobacter* spp. counts were reported as Log CFU/sample and statistical comparisons were estimated by Kruskal-Wallis analysis and post-hoc paired Wilcoxon's test comparison adjusted with Benjamin & Hochberg method (P < 0.05).

Results: Both methodologies showed similar quantification results in the two lines evaluated for PP and PC. There was a statistical difference (P<0.01) between both methodologies at R, PE, and W locations of 0.57 Log CFU/sample, 2.19 Log CFU/sample, and 1.49 Log CFU/sample for line 1. This may be related to the pathogen being present in concentrations lower than the limit of detection in certain sampling points where the microbial load was below 2.3 Log CFU/sample.

Significance: *Campylobacter* spp. may not be detected in bio-mapping studies when using quantification-only methodologies due to the low concentration; however, this does not mean that there is pathogen absence in the poultry rinsate. Therefore, detection methodologies need to be considered to supplement quantification data in order to minimize the risk of contamination in final products.

P2-91 Effect of Temperature, Incubation Time, and Growth Media on the Growth of *Salmonella enterica* Infantis

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Introduction: Salmonella Infantis is among the top serotypes of *Salmonella* associated with foodborne outbreaks in the US and has emerged as a problematic serotype in poultry production

Purpose: Previous studies with other *Salmonella* serotypes have necessitated the selection of appropriate growth temperature, nutrient medium, and incubation time before long-term *Salmonella* challenge studies in poultry

Methods: The growth of an *S*. Infantis strain in the MDR panel (CDC) was evaluated at two temperatures (37ºC – ambient bacterial growth temperature, 42ºC – poultry body temperature) in a general (Tryptic Soy broth; TSB) and a co-culture medium [TSB and deMan, Rogosa, Sharpe (MRS) broth at 1:1 ratio] for seven days. Tubes containing 10 mL TSB and co-culture medium were set at 37ºC and 42ºC for seven days. *Salmonella* populations were determined. Three samples per treatment were included, and the study was repeated two times (n=6/treatment).

Results: A completely randomized design *w*ith a factorial treatment structure was used in the study. Statistical analysis was done using the mixed procedure in R. *S*. Infantis grew to the fullest potential in both media at 37ºC, maintaining the populations until day 7 (*P*>0.05). Although *S.* Infantis reached similar populations in TSB at 42ºC, a reduced growth was noticed in the co-culture medium (6.5 log10 CFU/ml; *P*<0.05), maintaining the counts until day 7. This was different from our previous study with *S*. Heidelberg. Results indicate temperature influenced *S*. Infantis' growth at 42ºC in the co-culture medium, although it did not affect the growth at 37ºC in both media

Significance: This information is critical for determining ambient conditions for bacterial growth for the bird challenge studies, the success of which relies on the challenge dose, recovery rate, and time for necropsy and sample collection. The data will help develop interventions targeted against *S*. Infantis, a persistent problem for the poultry industry (USDA # 2020-67017-30787)

Poster

P2-92 Validation of the GENE-UP® Campylobacter Assay for the Detection of *Campylobacter coli*, *Campylobacter jejuni*, and *Campylobacter lari* in Poultry Using Hunt Broth

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Introduction: Campylobacteriosis, is the most common bacterial cause of diarrheal illness in the United States. *Campylobacter* are present in most warm-blooded animals, however human infection is most commonly associated with consumption of raw or undercooked contaminated poultry.

Purpose: Validation of a real-time PCR method using Hunt Broth compared to the USDA FSIS MLG 41.07 reference method for the detection of *Campylobacter* spp. from various poultry matrices as part of AOAC Performance Tested Methods℠ (PTM).

Methods: The candidate method was evaluated using paired test portions of chicken carcass rinsates (400ml), ground chicken (325g), and turkey carcass sponges (50cm^2) at three different target levels: 0 CFU/test portion (un-inoculated), 0.2-2 CFU/test portion (low) and ~2-5 CFU/test portion (high). Alternative confirmation was performed by direct plating onto CFA. All samples were confirmed regardless of screening result and all confirmed positive samples were identified using MALDI-TOF. Inclusivity and Exclusivity sets were also performed.

Results: Statistical analysis was performed using the Probability of Detection model. No statistically significant differences were observed between the candidate and the reference methods for all matrices. Uninoculated, low and high sets for all matrices had a dPOD of 0.00 (uninoculated and high confidence interval was -0.43, 0.43, low confidence interval for rinsates was -0.26, 0.26 and for ground and sponges was -0.28, 0.28). MALDI-TOF correctly identified to species level all confirmed positive samples. All 50 Inclusivity strains grown in Hunt were positive by the assay and all 30 Exclusivity strains grown in non-selective media were negative.

Significance: These data support the rapid and accurate use of GENE-UP *Campylobacter* for the detection of *Campylobacter* spp. from poultry requiring less time and subjectivity than traditional plating methods. VITEK® MS Prime is a simple and reliable identification option which can be performed directly from CFA further reducing the time to result.

P2-93 Quantification of *Salmonella* in Poultry and Pork Production-Related Samples Utilizing an Algorithm Based on the Output of a Loop Mediated DNA Amplification (LAMP) Based Bioluminescent Assay

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Introduction: Quantification of *Salmonella* in poultry and pork production processes enables characterization of the effectiveness of chemical and physical interventions to reduce the number of *Salmonella* in finished product. To date, most probable number (MPN) is still used as a method to determine the amount of a specific bacteria on a food sample, but the method is laborious and has a long time to result.

Purpose: To evaluate an algorithm to predict the concentration of *Salmonella* on a test sample utilizing short enrichment in non-proprietary medium and LAMP-based bioluminescent assay.

Methods: Algorithms to predict the concentration of *Salmonella* from 30-mL of carcass rinses were constructed utilizing the real-time output obtained after a test sample is enriched for 8 hours in buffered peptone water (BPW) ISO at 41°C and analyzed using a specific *Salmonella* LAMP-based bioluminescent assay. The algorithms were validated utilizing a minimum of 1100 samples and spiked in a range of 10^1-10^4 CFU/test portion. The concentration of *Salmonella* was queried from the algorithm and compared to the annotated value based on the inoculated concentration of *Salmonella* or MPN in naturally contaminated samples.

Results: The selected algorithm developed to predict concentration of *Salmonella* from carcass rinses was able to predict its concentration after 8 hours of enrichment. These predictions showed statistical equivalence ($p<0.05$) to microbial counts or MPN. Results have log difference of $\leq$0.5-1 CFU/test sample within dynamic range of 10^1-10^4 CFU/test portion.

Significance: The use of algorithms constructed with LAMP-based bioluminescent real time outputs may enable a faster and less laborious method to determine the concentration of *Salmonella* in pork and poultry matrices, allowing food processors to quickly evaluate the effectiveness of *Salmonella* control interventions.

P2-94 Correlation of Microbial Indicators and *Salmonella* Counts for Verification of Process Control in Commercial Pork Facilities in the United States

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❖ ***Developing Scientist Entrant***

Introduction: Under modernized pork inspection, processors are allowed to select their own indicator bacteria for process control as long as there are data to support the decision. It is important that the chosen indicator correlates to *Salmonella* behavior in order to ensure effective pathogen control.

Purpose: The purpose of this study was to identify microbial trends during processing through data-mining of two quantitative baselines of indicator microorganisms and *Salmonella*.

Methods: Samples were collected from two pork processing facilities throughout the course of 10 weeks, Plant A (N=650); Plant B (N=550). Swab samples were taken on the harvest floor and 2-pound product samples collected for trim and ground samples. The samples were chilled and shipped overnight to the ICFIE Laboratory at Texas Tech University. Aerobic Counts (AC), Enterobacteriaceae (EB), and generic *Escherichia coli* (EC) were evaluated using BioMérieux TEMPO®. *Salmonella* prevalence and enumeration was evaluated using the BAX® System Real-Time *Salmonella* and the SalQuant™ methodology. Microbial counts were converted to Log_{10}CFU/mL, Log_{10}CFU/g, and Log_{10}CFU/sample for statistical analysis. SPC parameters were developed for indicator microorganisms using the overall mean count ($\overline{X}$), the Lower control limit (LCL), and Upper control limit (UCL), based on the standard deviation, s and $\pm 3s$. Correlations were conducted using a Pearson correlation analysis.

Results: There was no major correlation of any microbial indicator to *Salmonella* quantification, p-value <0.05. However, indicator microorganisms were significantly reduced through the harvest floor for both plants, p-value <0.001. The polisher reduction for Plant A was 1.24, 1.71, and 2.13 LogCFU/mL for AC, EB, and EC, respectively. The bio-furnace reduction for Plant B was 2.56, 1.87, and 1.72 LogCFU/mL for AC, EB, and EC, correspondingly.

Significance: While it may be practical and effective to utilize microbial indicators for process control, *Salmonella* quantification may be more beneficial when validating the impact of antimicrobial treatments or processes for *Salmonella* control.

P2-95 Evaluation of Swine Hindgut-Mucosal Microbiome in Association with Food Safety

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Introduction: The swine large intestine has widely been consumed by people worldwide. Even though swine large intestine contains a variety of microorganisms that might cause food borne illnesses, there is limited information on the public health risk of microorganisms in the swine large intestine.

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Purpose: The purpose of this study was to evaluate the public health risk of microorganisms in the swine hindgut mucous membrane using a metagenomics approach.

Methods: A total of 18 swine large intestine samples were collected from the pigs. Total DNA from the fresh swine hindgut mucous membrane was extracted, and the 16S rRNA gene V5-V6 hypervariable regions were sequenced using the Illumina MiSeq platform. Sequencing data was analyzed using MicrobiomeHelper and QIIME2 workflow. Statistical analysis was performed using STAMP.

Results: The average number of sequence reads generated per swine hindgut mucous membrane was 162,653. Alpha diversity indices (Chao1 891, Shannon 6, Simpson 0.968) of the swine hind-gut mucous microbiome indicated highly diverse bacterial richness. Taxonomic classification of the sequences showed that phyla Bacteroidetes and Firmicutes were the most abundant at the phylum level. Even though the relative abundance of Campylobacterota was low, it was detected in the samples. At the genus level, potential pathogens, such as *Clostridium, Helicobacter* and *Pseudomonas* were identified in the swine hind-gut mucous membrane. Overall, our data showed that the metagenomics approach could be used to evaluate the public health risk of microorganisms in the swine hindgut mucous membrane.

Significance: Our data showed that the swine large intestine can harbor the potential pathogens, such as *Clostridium, Helicobacter* and *Pseudomonas*. Therefore, extra care is required to cook swine large intestine for human consumption to reduce the public health risk of microorganisms in the swine hindgut mucous membrane.

P2-96 Use of a Doehlert Matrix to Identify Antimicrobial Combinations on the Surface of Raw Pork Meat Against *Salmonella* spp.

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella* spp. represent a food safety threat to the pork industry. Common antimicrobial practices include spraying organic acids directly onto the pork carcasses. Many studies are published every year that address novel antimicrobial interventions against *Salmonella* spp. on pork. These studies typically use full factorial designs to identify combinations, which is time-consuming and it is expensive.

Purpose: Use a Doehlert Matrix (DM) experimental design to more efficiently study the effect that individual and 2-way combinations of antimicrobials have on the growth of *Salmonella* on raw pork meat, this approach can accommodate new compounds as they become relevant.

Methods: The selected independent variables were: lactic acid, formic acid, cumin, peppermint, clove, and spearmint. The designed DM resulted in 42 experiments, 1 central point in triplicate, and 1 positive control in duplicate. The designed treatments were surface applied to *Salmonella*-inoculated miniaturized pork disks (0.55 g), which were incubated at 4°C for 24 h. Bacterial cell counts were enumerated. The model response was the change in *Salmonella* cell counts after 24 h compared to the inoculum (in log CFU/g), this response was used to build a second-order model using linear regression.

Results: The model identified 5% lactic acid and 3% formic acid as significant factors, a 1.5 and 2.2 log CFU/g *Salmonella* reduction, respectively. The regression was significant (p-value<0.0001) with a residual standard deviation of 0.12 log CFU/g. An analysis of 2-way interactions showed 5% lactic acid-1% peppermint and 3% formic acid- 1% spearmint to yield a 0.58 and 0.60 log CFU/g *Salmonella* reduction, respectively. The remaining 2-way combinations showed non-significant reductions (p-value>0.10).

Significance: The use of this tool will allow academia and industry to build on the already existing knowledge on effective antimicrobials compounds and their combinations in a fast and cost-effective way.

P2-97 Evaluation of the *Escherichia coli* Population during a Chicken Slaughter

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Introduction: *Escherichia coli* is an indicator of quality for the animal products industry, and its monitoring during the slaughter process is essential to assess the efficiency of hygienic procedures throughout the slaughter stages.

Purpose: To verify the efficiency of the technological stages of broiler slaughter in the logarithmic reduction of *E. coli* throughout the process.

Methods: We carried out the study in a large slaughterhouse for export, with a capacity to slaughter over 500,000 birds per day, located in Brazil. For 10 weeks, we analyzed broilers in seven points: pre-scalding; post-scalding; post-plucking; post-evisceration before CCP1B; after CCP1B; after the final shower and post-chiller, in a total of 490 carcasses (70 carcasses per point). We performed the quantification of *E. coli* according to AOAC Official Methods 998.08 using Petrifilm EC. We expressed the results in log CFU/g and used analysis of variance to compare means; differences were determined by the Tukey test (level of 0.05).

Results: During the process, we observed reductions in counts, with emphasis on the significant reduction of 2.413 log CFU/g between pre-scalding and post-scalding. The contamination remained stable in the later stages, falling again significantly after CCP 1B and with a total reduction in the process of 4.473 log CFU/g. We also evaluated the effect of variables such as the slaughter shift, where the *E. coli* counts were 0.5 log CFU/g higher in the 2nd shift than in the 1st shift ($P<0.05$). The fasting time above 12 hours also provided more contamination ($P<0.05$).

Significance: There was a significant reduction in *E. coli* counts throughout the process, and the scalding and CCP1B steps were the most efficient for the reduction of *E. coli*, and the slaughter shift and fasting time contributed to the highest or lowest contamination observed. Acknowledgments: CAPES, CNPq.

Poster

P2-98 Prevalence, Serovars, and Antimicrobial Resistance of *Salmonella enterica* in Hatchling Chicks Sold in Vermont Agricultural Supply Stores

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Introduction: Forty percent of chicks sold at agricultural supply stores in Vermont were previously found to have *Salmonella enterica,* but serovars and antimicrobial resistance genes were not assessed.

Purpose: The purpose of this study was to determine the prevalence, serovars, and antimicrobial resistance of *S. enterica* in chicks sold at Vermont agricultural supply stores

Methods: We collected shipping pads and/or soiled bedding at 10 stores and one independent hatchery in VT from March thru July 2022, recording hatchery, breed, and age of chicks. 25-gram samples were enriched in buffered peptone water for four hours at 37° C, followed by Rappaport-Vassiliadis and Tetrathionate enrichments for 24 hours at 42°C and 37°C, respectively. Positive samples were confirmed with XLT4 or R&F laboratories Salmonella (Nontyphoidal) Chromogenic Plating Medium agar at 37 and 35 °C, respectively for 48 hours. Identification was confirmed *hilA* PCR. 26 isolates from 2021-2022 were sequenced using the Oxford Nanopore MinION PCR-Barcoding protocol, assembled with Flye (v.2.6) and polished using Medaka (v.1.6.0). Serovars were determined using SISTR (v.1.1.1) and SeqSero (v.1.2). Antimicrobial resistance (AMR) genes were identified using ResFinder (v.4.1).

Results: 79/195 (40.5%) samples were positive for *S. enterica*. 45.1% of samples from hatcheries enrolled in the National Poultry Improvement Plan (NPIP) were positive, compared to 33.9% from hatcheries not enrolled in NPIP. Serovars for the 26 isolates sequenced were as follows: Enteritidis (12), Cerro (4), Seftenburg (4), Kentucky (3), and one each of Braenderup, Infantis, and Mbandaka (1). Only *Salmonella* Seftenburg isolates had AMR genes; two isolates had β-lactamase genes (*blaTEM-104, blaTEM-1b,* and *blaACT-12*), and one each had *tetJ, fosA2, qacE, Sul1, ant(2")-1a*, and *aadA2.*

Significance: *S. enterica* was found in 40% of chicks from agricultural supply stores in 2022, similar to 2021. AMR genes were rarely found, but serovars present in chicks match those involved in U.S. salmonellosis outbreaks associated with live poultry.

P2-99 Microbiological Acceptability of Chicken Breast Meat and the Proliferation of Antimicrobial-Resistant *E. coli* in Broiler Chickens in Lebanon

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❖ ***Developing Scientist Entrant***

Introduction: Several foodborne disease outbreaks have been reported and pathogenic microorganisms have been detected in food, including poultry meat, in Lebanon. In addition, antimicrobial agents are extensively used in animal farming to control infections and increase productivity.

Purpose: The aims of this study were to identify and quantify bacterial contamination and antimicrobial susceptibility associated with poultry meat in Lebanon.

Methods: A total of 151 skinless chicken breasts were purchased from 51 retail markets (N= 25) and butcheries (N= 26) in Lebanon. Also 549 fecal samples were collected from 6 farms across the country. To enumerate fecal coliforms and *E. coli*, 25 grams of skinless chicken breasts were homogenized for 60 seconds in a stomacher. Serial dilutions were prepared and of the dilutions were plated on RAPID'*E. coli* 2 Agar and incubated at 37°C for 24 hours under aerobic conditions to count colony forming units. Antimicrobial susceptibility testing using disk diffusion assay was performed on *E. coli*. Antimicrobial resistance was determined by measuring the diameter of the zone of inhibition around each antibiotic disc, and then interpreted based on breakpoints available from the Clinical and Laboratory Standards Institute and from the European Committee on Antimicrobial Susceptibility Testing.

Results: The prevalence of fecal coliforms and *E. coli* on skinless chicken breast samples was 100% and 79.5%, respectively. According to LIBNOR standards (acceptable limit for fecal coliforms < 5000 CFU/g), 147 out of 151 (97.4%) skinless chicken breast samples were deemed unacceptable. The *E. coli* isolates were highly resistant to ampicillin (69%), tetracycline (68%), ciprofloxacin (59%), and chloramphenicol (51%). *E. coli* isolated from fecal samples were highly resistant to ampicillin (95%), tetracycline (87%), chloramphenicol (83%), trimethoprim-sulfamethoxazole (75%), and ciprofloxacin (71%). Around 70% of *E. coli* isolates from skinless chicken breast samples and 95% from fecal samples exhibited MDR.

Significance: These findings highlight the need to monitor the safety and microbiological quality of poultry meat in Lebanon.

P2-100 A Systematic Review and Meta-Analysis of Interventions to Reduce *Salmonella* and *Campylobacter* during Chilling and Post-Chilling Stages of Poultry Processing

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Introduction: *Salmonella* and *Campylobacter* are among the most common causes of foodborne illnesses worldwide. Processing interventions, such as chilling and post-chilling, are vital to reducing contamination on poultry products before they reach consumers.

Purpose: The aim of this systematic review and meta-analysis was to determine the impact of chilling and post-chilling interventions used in poultry processing on the population and prevalence of *Salmonella* and *Campylobacter*.

Methods: A comprehensive search of the literature published between 2000 and 2021 was conducted in the databases Web of Science, Academic Search Complete, and Academic OneFile. Studies were included if they were in English and investigated the effects of interventions against *Salmonella* or *Campylobacter* on whole carcasses or parts during the chilling or post-chilling stages of poultry processing. Random-effects meta-analyses were performed using the "meta" package in the R programming language. Subgroup analyses were assessed according to outcome measure reported, microorganism tested, processing stage assessed, and chemical treatment used.

Results: The results included 41 eligible studies. Nineteen studies reported results of 30 separate interventions against *Salmonella*, and 31 reported results of 50 separate interventions against *Campylobacter.* No significant difference ($P > 0.05$) was observed when comparing interventions against *Salmonella* and *Campylobacter* or when comparing chilling times. For analyses examining antimicrobial additives, peroxyacetic acid (PAA) had the largest reduction against *Salmonella* concentration regardless of chilling time ($P < 0.05$). PAA also had the largest reduction against *Campylobacter* population and prevalence during primary chilling ($P<0.01$). Air chilling showed a lower reduction for *Campylobacter* than the immersion chilling interventions ($P<0.05$).

Significance: Chilling time and antimicrobials used during poultry chilling and post-chilling had varying effects depending on the pathogen and outcome measure investigated. High heterogeneity and low study numbers in most analyses suggest that more high-quality research is needed to corroborate the results.

P2-101 Antibacterial Efficacy of Phage Against Antibiotic-Resistant *Campylobacter jejuni* in Chicken Skin

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Introduction: Campylobacteriosis is a leading foodborne illness associated with *Campylobacter jejuni* contamination during slaughtering and processing of poultry products.

Purpose: This study aims to characterize *Campylobacter* lytic phages and determine their biocontrol efficacy against tetracycline-resistant *C. jejuni* CA-2046 on poultry carcass.

Methods: The efficacy of individual and phage cocktail, consisting of four Group (III) *Campylobacter* lytic phages (CPS1, CPS2, F198, WSS1), to control *C. jejuni* CA-2046 in broth for 24 hours at a multiplicity of infection (MOI) of 10 and 1000 using plate count and turbidimetric approach was used. Phage efficacy was tested on chicken skin using an MOI of 10,000. The samples were incubated aerobically at 4°C, 15°C and 25°C at specific time points.

Results: Amongst the four phages, the highest log reduction was achieved by CPS2 at MOI 10 and CPS1 at MOI 1000. At MOI 10, the four-phage cocktail and CPS1 achieved approximately 1.77 log CFU/mL reduction in bacterial count. At MOI 1000, the four-phage cocktail showed a 2.09 log CFU/mL reduction, almost half of that observed when individual phages were tested. At MOI 1000, two phage cocktail (CPS1 and CPS2) showed double the reduction than four-phage cocktail, however, the reduction of both cocktails was lower than that for CPS1. Hence, CPS1 was selected for the biocontrol experiment in chicken skin. A 1-1.4 log reduction was observed at between 0h and 24h at all three temperatures with greatest reduction observed within 6h of phage application. CPS1 was able to significantly maintain *C. jejuni* reduction after 24h at all temperatures.

Significance: This study shows a possible antagonistic activity of campyphages when present in a cocktail. Furthermore, it illustrates the potential of phages to be used as biocontrol agents to mitigate the risk of *Campylobacter* in raw poultry products.

P2-102 Phage-Active Packaging: Phage-Loaded Electrospun Nonwoven with Antimicrobial Properties Against *Salmonella* enteritidis

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Introduction: *Salmonella* is one of the most prevalent food-borne bacterial pathogens with the highest incidence reported in poultry. Lytic phages have been proposed as natural antimicrobials in different settings including food packaging. Electrospinning is a technique for producing submicron fibers, beneficial for the encapsulation and controlled release of bioactive compounds.

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Purpose: To evaluate the antimicrobial effect of a phage cocktail encapsulated in electrospun poly(ethylene oxide) (PEO) nonwovens against *Salmonella* Enteritidis *in vitro* and in chicken meat.

Methods: A broad spectrum *Salmonella* phage cocktail comprised of five phages was used in this study. The phage cocktail was dispersed in 12% (w/v) PEO aqueous spin dope solution and then electrospun at 30 kV using a free-surface electrospinning machine. To control the release of the phage particles, the PEO phage-loaded nonwoven layer was sandwiched between two layers of poly(lactic acid) and PEO blend. Finally, the antimicrobial effect of the phage-loaded composite nonwoven was evaluated against *Salmonella* Enteritidis in TSB medium and chicken exudate. A final concentration of 5 log CFU/ml was treated with phage fibers (5 log PFU/ml) or the equivalent of free phage. The bacterial reduction was evaluated at three temperatures (25, 15, and 4°C) for up to 72 h.

Results: An average of one log PFU/mg reduction post-electrospinning was observed for all phages in the cocktail. Composite nonwovens containing 6 log PFU/mg were obtained. When placed in a buffer solution, the composite nonwoven gradually released the phage, reaching the maximum release at 2 h. The phage-loaded nonwoven showed the same antimicrobial effect compared to free phage with 7, 5, and 3 log CFU/ml reduction at 25, 15, and 4°C, respectively in both media evaluated. The antimicrobial effect of the phage-loaded nonwoven in chicken meat is underway.

Significance: This study demonstrates the potential of using phage-loaded nonwovens in food packaging material to control the growth of *Salmonella*.

P2-103 Low-Cost, Printed, Electrical Gas Sensors for the Assessment of Spoilage in Chicken Fillets.

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Introduction: The use-by date does not always reflect the actual level freshness of food. The integration of disposable sensors in the packaging, compatible with digital platforms that can provide real time information about the state of freshness could be a more precise alternative.

Purpose: Investigation of the sensors' efficiency to estimate the microbial populations in chicken fillets.

Methods: Chicken fillets (n=120) were obtained from poultry industry "KOTINO". The samples were placed on polystyrene trays, packaged in both aerobic and MAP (70/30, N_2/CO_2) conditions and stored at 4 and 8 °C for certain days. A sensor device, connected to a smartphone, was integrated in each package. Microbiological analysis for the estimation of total aerobic counts (TAC) and specific meat spoilage bacteria, was performed. The data acquired from sensors and the microbial data were used in PLS regression models for the prediction of microbial counts in MAP and air-packaged samples as well as in all samples regardless of packaging condition. The root mean squared error (RMSE) and the coefficient of determination (R^2) were used to evaluate the models' performance.

Results: Microbial populations were in the range of 4.50 to 9.00 log CFU/g, while the growth rate and the microbial profile were differentiated in MAP samples compared to the air-packaged ones. Concerning sensors' performance, PLS models could satisfactorily predict TAC in aerobic conditions (R^2, 0.88; RMSE, 0.32). The model presented poor performance in MAP conditions (R^2, 0.30; RMSE, 1.22), while the model developed, regardless of the packaging conditions, exhibited good results (R^2, 0.74; RMSE, 0.66).

Significance: The proposed, near-zero-cost, sensors can estimate the microbial populations by detecting the level of water-soluble gases and give real time information about the freshness of chicken.This work has been funded by the project DiTECT (861915) while the sensors were provided by Blakbear.

P2-104 Fluorescence Imaging System for the Detection of Fecal Contamination on Chicken Carcasses

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❖ ***Developing Scientist Entrant***

Introduction: Fecal contamination has a zero-tolerance policy in the poultry processing industry that is enforced by the USDA-FSIS to prevent the spread of foodborne pathogens to consumers.

Purpose: The objective for this study is to assist in the identification of fecal matter on processed chicken carcasses that cannot be seen with the human naked eye using a fluorescence imaging system through still images.

Methods: A total of fifty chicken carcasses were collected from a processing plant. Four different types of fecal contamination (digesta, small intestine, large intestine, and ceca) were used to place on the breast of whole-chicken carcasses for comparison. Chicken carcasses were placed in an ambient light-free box and observed with a spectral handheld imaging system. Images were collected for further image analysis using Convolution Neural Network deep learning (CNN-DL) algorithms. CNN model was developed on Google Collaboratory platform using CUDA 11.2 and Pytorch machine learning framework with 3 ConvNet layers. Adam Optimizer (learning rate =0.001, weight decay = 0.0001) with cross entropy loss function was used for training of model with 20 epoch. And a simple, "click-and-play" Azure Custom Vision, a machine learning algorithm, was used to classify fecal contamination based on its source.

Results: Developed CNN-DL algorithm was able to identify the fecal matter presence on the chicken carcasses with a testing accuracy of 63.6%. In a comparison analysis, Azure Custom Vision was able to differentiate between the fecal source images on chicken carcasses, however the precision for ceca, proventriculus and small intestine was 71.4, 66.7 and 50% while large intestine has a precision of 0%. Improving image quality and number would be beneficial to build robust classification models.

Significance: Spectral imaging system can be deployed in poultry processing plants to detect fecal contamination and ultimately improve food safety.

P2-105 Multidrug-Resistant *Campylobacter jejuni* and *Campylobacter coli* Isolated from Chicken Meat in the Peruvian Amazon

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Introduction: Increasing levels of fluoroquinolones and macrolide resistant *Campylobacter jejuni* and *C. coli* pose an emerging health threat and applied clinical problem.

Purpose: We examined and compared genomic determinants of antimicrobial resistance in Campylobacter isolated from chicken meat sampled in Iquitos, Peru.

Methods: Chicken meat was systematically sampled from markets for 12 months. *Samples were pre enriched in Bolton Broth and cultured in* Columbia Blood Agar Base supplemented with 5% lysed horse blood. Phenotypic antimicrobial susceptibility patters were assessed using standard disc-diffusion (Kirby-Bauer) methods. Genomes were sequenced using an Illumina MiSeq generating 250 bp paired-end reads, and antimicrobial resistance genes were identified using standard databases and manual examination of genomes.

Results: Between November 2021 and December 2022, a total of 456 meat samples were cultured, 235 Campylobacter isolates were obtained, and 200 Campylobacter isolates were sequenced. Twenty percent (47/234) of isolates were azithromycin resistant and 89.4% (210/235) were ciprofloxacin resistant. These rates statistically significantly lower than for Campylobacter isolates obtained from children with gastroenteritis and asymptomatic carriage sampled during the same time period. Genomic determinants of fluoroquinolone resistance included the Thr86Ile point mutation in the gyrA gene, while the azith-

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romycin resistance was most frequently attributed to the A2075G mutation in 23S rRNA. *RE-cmeABC* was significantly associated with multidrug resistance (p<0.001). MLST and phylogenetic relationship of C. jejuni and C. coli isolates is presented.

Significance: High levels of macrolide and quinolone resistant Campylobacter isolates were identified in chicken meat in Iquitos, Peru, posing an important public health threat. There is evidence to support that efforts should tailored towards reducing antibiotic use as growth promotion in the poultry sector and slaughtering practices are improved to reduce Campylobacter contamination of poultry meat.

P2-106 Evaluating the Efficacy of Peroxyacetic Acid Treatment Variables Against *Salmonella* on Chicken

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Introduction: Peroxyacetic acid (PAA) formulations are commonly used during poultry processing to reduce microbial loads, including *Salmonella*. Wash solutions containing PAA are used at varying concentrations during processing and processors may adjust PAA solutions to a pH above 7 to preserve the product quality. There is little research on how variation in temperature, pH, and contact time in combination with PAA concentration affect the survival of *Salmonella*, and such information could improve the efficacy of PAA treatments during poultry processing.

Purpose: This study investigated the combined effects of PAA concentration, pH, temperature, and contact time on the reduction of *Salmonella* on chicken wings.

Methods: Chicken wings were inoculated with a six-strain cocktail of antimicrobial-adapted *Salmonella enterica* (one strain, each, of serotypes Heidelberg, Kentucky, Typhimurium, Enteriditis, and Infantis) to an initial concentration of ~5.5 log CFU/g. The factorial experimental design consisted of five PAA concentrations (0, 50, 200, 500, and 1000 ppm), three pHs (unadjusted, 6.0, and 8.5), two temperatures (4 and 27°C), and two contact times (10 and 20 s). The surviving *Salmonella* populations were enumerated by direct plating and reported as log CFU/ml of the rinsate.

Results: The efficacy of PAA in reducing the population of *Salmonella* on chicken wings was dependent on the concentration and the temperature of the PAA solutions. Treatment conditions of 4°C resulted in a lower population ($P \leq 0.05$) of recoverable *Salmonella* at all tested PAA concentrations, compared to treatment at 27°C. Maximum reduction of ~0.9 log CFU/ml was seen at 1000 ppm PAA and 4°C. There were no significant effects ($P > 0.05$) of pH or contact time.

Significance: Treatment conditions, such as temperature, can impact the effectiveness of PAA used as an antimicrobial treatment during poultry processing, and the results from this study can provide useful insights to pH adjustments and the lack of antimicrobial efficacy.

P2-107 Microbiological Risks in Inspected and Uninspected Poultry in British Columbia, Canada

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Introduction: Regulatory changes (2021) allow poultry that is not inspected during slaughter to be sold in stores and restaurants with a Farmgate+ license in the province of British Columbia.

Purpose: A microbiological survey was conducted to assess differences between uninspected (Farmgate+) and inspected poultry.

Methods: Search strategies for poultry farms used a combination of consulting on-line registries of inspected, licensed (federal, provincial) operators and on-line searches using Facebook, Google, farmers' market sites and word of mouth. Raw, fresh or frozen poultry were obtained directly from farms, butcher shops and retail locations in BC between August and December 2022. Poultry was designated as inspected, uninspected or unknown based on labelling and presence on registries. Samples were tested using Health Canada compendium methods for *Campylobacter* (MLG-41.06), *Listeria* (MFHPB-30), *Salmonella* (MFHPB-20), *Staphylococcus aureus* (MFHPB-21) and *E. coli* (MFHPB-34). Chi-square contingency analysis tested differences between proportions of bacteria and categories of inspection.

Results: 55 samples were collected: 34 inspected (18 federal, 16 provincial), 9 uninspected (Farmgate+) and 12 unknown. Most poultry were found via on-line search strategies (74.5%) in comparison to on-line registries (25.4%). Samples tested included chicken (78%) and turkey (22%). Microbiological tests found *Campylobacter* in 38.2%, *Listeria* spp. in 32.7% with *L. monocytogenes* in 5.5%, generic *E. coli* in 34.5%, *Salmonella* in 3.6%, with no *S. aureus* detected. No significant differences were found (p<0.05) between inspection categories.

Significance: Improper labelling on samples purchased make it difficult to ascertain where slaughter was occurring. Although not significant, a higher proportion of *E.coli* was found in unknown poultry sources, and no *Listeria* spp. were detected in uninspected, Farmgate+ samples.

P2-108 Use of Bax System Polymerase Chain Reaction to Detect S*almonella* isolates from Pre-Harvest Floors from a Beef Facility

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Introduction: *Salmonella* is a foodborne pathogen commonly found in cattle feces, hides, carcasses, and harvest production environments. Many factors impact *Salmonella* presence in animals including breed, age, the time of feed and water withdrawal, geographical location, and season. The incoming contamination can impact the final product contamination. Therefore, it is essential to develop rapid technologies to detect pre-harvest prevalence and concentration of pathogens to inform risk management activities in the beef industry.

Purpose: To evaluate the performance of BAX system PCR as a screening tool for pre-harvest *Salmonella* contamination compared to conventional culture-based methods.

Methods: A total of 160 *Salmonella* presumptive isolates were collected from the pre-harvest floors of a beef facility located in the Midwestern region of the United States. *Salmonella* in samples was detected and enumerated by BAX System PCR assay and SalQuant. In parallel, each pre-enriched sample was subjected to culture isolation following the FDA-BAM method. The typical colonies were confirmed by a rapid *Salmonella* latex agglutination test. Positive or negative culture isolates for each sample were also confirmed by BAX System PCR assay. Results from both methods were compared.

Results: *Salmonella* was detected in 99.3% (159/160) of samples with a mean estimated count of 2.12 ± 0.93 Log CFU/m as screening tests according to the BAX (rapid) results. There was 95.6% (152/159) and 93.7% (149/159) agreement between the BAX system assay and conventional culture method compared to the screening tests, respectively. According to this data, the BAX method is also suitable for pre-harvest samples, which originally was developed for product samples.

Significance: This data supports this validated PCR method detects *Salmonella* strains with a fraction of the time required for the reference conventional methods. Detecting pathogens during pre-harvesting stages and knowing the incoming loads will allow the facility to implement interventions in the harvesting production environments to increase food safety.

P2-109 Development of Copper Alginate Beads as a Slow-Release Delivery System to Reduce Pathogen Shedding in Swine

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Introduction: Copper has proven to be an effective antimicrobial agent. Copper-alginate beads (CB) were designed and evaluated as a pre-harvest intervention for finishing pigs. The formulated beads could allow copper to remain longer in the gastrointestinal tract of animals, reach deep into both the foregut and hindgut, and reduce the concentration of pathogenic bacteria.

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Purpose: To investigate the potential of CB to reduce pathogenic microorganisms, and to evaluate the effectiveness of encapsulating to slow the copper release rate.

Methods: CB were tested on a 10^6 CFU/ml *Salmonella* cocktail and compared with copper sulfate pentahydrate powder (CS) as a non-encapsulated form of copper. Three test tubes containing 5ml of *Salmonella* cocktail were tested with the copper treatments (CB, CS, and no treatment). CB and CS were placed inside the cocktail and left for one hour. After exposure time, the *Salmonella* cocktail was removed, leaving the copper treatment in each test tube. 5 ml of a new untreated cocktail was added to each tube containing the original treatments. This was repeated for six hours. Upon removal of the bacterial solution, 1 ml aliquot was saved at each time point to test the copper concentration remaining in the bacterial solution by flame atomic absorption spectroscopy. A pairwise t-test was used to assess *Salmonella* concentration between copper treatments.

Results: Significant *Salmonella* reduction was observed over time with CB treatments compared to CS treatments. The CS dissolved in the solution; therefore, it was removed with the bacterial cocktail after the first hour. No CS was present the subsequent exposure times to reduce *Salmonella*. The encapsulated copper was proven to be released slowly (up to 5 hours) in a *Salmonella* cocktail.

Significance: Results suggest that CB reduces microbial populations, and encapsulation is potential means for the delivery of antimicrobial copper to the animal hindgut.

P2-110 Experimental Evaluation of Tylosin Use for the Prevention of Liver Abscesses in Beef Cattle on Bacterial Resistance to Critically Important Antibiotics for Human Use

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Introduction: Although tylosin, a veterinary only macrolide used to prevent liver abscess in feedlot cattle, was shown to select for macrolide resistant Gram-positive bacteria, its influence on Gram-negative foodborne bacteria is unknown.

Purpose: Evaluated the effect of continuous in-feed tylosin use on the concentration and prevalence of tetracycline resistant (TET^r)-, 3rd generation cephalosporin resistant ($3GC^r$)-, and extended spectrum beta-lactamase producing (ESBLs)-*E. coli* in feedlot cattle.

Methods: A cohort of weaned calves with 10 animals/group were randomized to receive tylosin supplemented mineral (Tylosin group) or non-supplemented mineral (Control group). Fecal samples were collected approximately monthly for the entire feeding period of one year. Pen surface and feed samples were also collected. Samples were cultured on antibiotic supplemented media. Enumeration and binary outcomes were analyzed by multilevel mixed effects linear regression or logistic regression, respectively using treatment and days on feed (DOF) as factors.

Results: Tylosin did not significantly (P>0.05) affect total and TET^r fecal *E. coli* concentrations, although their concentrations significantly (P<0.001) increased over time. Tylosin significantly (P=0.006) increased the fecal prevalence of ESBLs-*E. coli* with no significant (P=0.4) effect on the fecal prevalence of $3GC^r$ *E. coli*. Fecal prevalence of $3GC^r$- and ESBLs-*E. coli* significantly (P<0.001) increased over time. $3GC^r$ and ESBLs-*E. coli* were not detected from feed samples; they were detected from pen-surface samples with no significant (P>0.05) tylosin effect.

Significance: In-feed tylosin use to prevent liver abscess in feedlot cattle, although known to select for antimicrobial resistant Gram-positive bacteria such as enterococci, did not select for antimicrobial resistant Gram-negatives including ESBLs-producing Enterobacteriaceae, considered as a serious public health threat by the U.S. CDC. However, the study indicated that feedlot cattle production setting gradually increases the levels of *E. coli* resistant to the critically and/or important antibiotics for public health, indicating an increased risk of their dissemination beyond the beef cattle production setting.

P2-111 Litmichic: A Direct-Fed Microbial That Can Limit the Development of Antimicrobial Resistant *Salmonella*

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Introduction: Direct fed microbials, such as probiotics, have been shown to enhance productivity and reduce pathogen loads in the gastrointestinal tract, however there is still significant concerns about their safety, and no probiotic has been shown to stop the transfer of antimicrobial resistance (AMR) between bacterial species.

Purpose: Our objective was to develop a probiotic that is safe and that can reduce the population of antimicrobial resistant food-borne pathogens.

Methods: We performed in-silico and in-vitro studies to determine the safety and efficacy of a probiotic composed of four *Bacillus velezensis* strains (named Litter and My Chickens - LitMiChic). To determine if the probiotic could reduce the development of AMR in *Salmonella*, we performed conjugative transfer assays in the presence/absence of the probiotic. We used *E. coli* strains (n = 3) that harbored transferable AMR genes (*aadA1, aac(3)-Via, blaCTX-M1, sul1, sul2, and tetA*) as donors and *Salmonella* Heidelberg strains (n = 3) that did not carry AMR genes as recipients.

Results: In-silico whole genome sequencing analysis revealed that the probiotic strains are safe as they did not harbor AMR or virulence genes that could be exchanged with other bacterial species. Adding the probiotic to solid agar significantly (P< 0.05) reduced the rate of transfer of AMR genes from *E. coli* to *Salmonella* Heidelberg by 99.98 ± 0.0001%. Additionally, total *Salmonella* and *E. coli* population were reduced by ~ 3 logs.

Significance: These results suggest that LitMiChic has the characteristic of a probiotic that can be used for AMR and food-borne pathogen reduction, and further studies are underway to determine the efficacy of LitMiChic in broiler chickens.

P2-112 Fecal Shedding of Shiga Toxin-Producing *Escherichia coli* in a Small Cattle Feed Yard in Close Proximity to Leafy Greens

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Introduction: Shiga-toxin producing *Escherichia coli* (STEC) infections cause human illness ranging from mild diarrhea to hemorrhagic colitis and life-threatening hemolytic uremic syndrome. Domesticated cattle are considered the primary animal reservoir of *E. coli* O157:H7 with transmission through consumption of undercooked meat and dairy products; but, the mechanism(s) of transmission between cattle operations to other foodborne sources such as fresh produce remain elusive.

Purpose: Survey STEC prevalence in finishing steers at a 250-head university feedlot in the southwestern desert, and measurement of *E. coli* levels at different distances between the cattle pens and adjacent leafy green produce fields.

Methods: Fresh fecal samples were collected from cattle pens from April to November 2020. Samples were cultured for STEC by selective enrichment and plating. Presumptive positive colonies were confirmed and sequenced to identify the predicted serotype. Romaine lettuce and spinach samples were sampled at different distances (200 to 800 feet) at harvest from an adjacent field.

Results: Overall, 33 (12%) and 44 (16%) of 270 cattle fecal pen samples were positive for *E. coli* O157:H7 and non-O157, respectively. Among 54 non-O157 STEC isolates from unique samples, the majority of predicted serotypes included -:H10, O116:H9, O109:H10, O171:H2, O55:H12 O55:H12, or O26:H11. The odds of finding non-O157 STEC was 2.3 times higher in the fall compared with Spring (p=0.049). STEC was not detected in adjacent leafy green samples at harvest, but generic *E. coli* was significantly higher in samples collected 200 feet from cattle pens (5.5 x 10^3 MPN/g) compared with produce collected 300 to 800 feet (<1MPN/g).

Significance: STEC is prevalent even in a relatively small cattle feed yard and can be seasonal. Movement of *E. coli* from cattle pens to adjacent produce fields may be limited to short distances (<300 feet), which can inform guidelines for set-back distances from low density livestock operations near leafy green fields.

P2-113 Assessing Changes in Enteric Pathogen Population during Protozoan Challenge in Turkey Poults

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❖ ***Developing Scientist Entrant***

Introduction: *Cochlosoma anatis*, a flagellated protozoan parasite of turkeys, causes runting, flock non-uniformity, diarrhea, and depression with high flock morbidity and is commonly associated with enteric co-infections.

Purpose: This study aimed to characterize differences in populations of *Escherichia coli*, *Clostridium perfringens*, and *Salmonella spp.* relating to infection with *C. anatis* at different days of age in turkey poults.

Methods: Two hundred 1-day-old poults were placed in 20 isolation cages (10 birds/cage). With 4 treatments, 5 cages per treatment were challenged with $5x10^5$ *C. anatis* cells/mL on day 0, 7, and 14 of the trial with a non-infected negative control (NC). At D28, the cecal tonsil and ileum were aseptically removed from up to 3 birds/cage for bacterial and *C. anatis* quantification, respectively. *E. coli*, coliforms, and *Salmonella spp.* were reported in CFU/g using a most probable number (MPN) assay using Colilert and TT broth following USDA MPN guidelines. *C. perfringens* was quantified by plating on tryptose sulfite cycloserine agar and colony counting reported in CFU/g. *C. anatis* was quantified by microscopic counting per ileal section sampled. Statistical analysis was performed using a one-way ANOVA and Duncan's multiple range test with mean separation in SAS 9.4 with α=0.05.

Results: The results from this study showed no significant differences between the tested bacteria and infection time. However, there was a noticeable drop in bacterial concentration across all bacteria tested, with the lowest amounts relating to earlier infection with *C. anatis*. *Salmonella spp.* was scarcely recovered overall.

Significance: These results indicate that the concentrations of bacteria in the poult intestines did not change due to infection with *C. anatis.* The drop in population may be due to watery diarrhea expelling additional pathogenic bacteria into the environment. Interactions may also exist between *C. anatis* or the immune system and the bacteria, causing bacterial death resulting in lower intestinal concentrations.

P2-114 What Affects the Survival of *E. coli* in Midwest Agricultural Soils?

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Introduction: Due to an increasing number of foodborne outbreaks associated with biological soil amendments and produce, there is a need to understand how soil texture and nutrients influence pathogen growth.

Purpose: The objectives of this study were to evaluate the effect of soil texture (Clay loam, Silt Loam, Loamy Sand) and nutrients (Carbon, Nitrogen, Phosphorus) on *Escherichia coli* (*E. coli)* survival.

Methods: Clay loam (CL), silt loam (SIL) and loamy sand (LS) soils were inoculated with an *E. coli* cocktail (4.67 log CFU/gm dry soil) and incubated at 37°C. Moisture was adjusted to 25% for CL, 20% for SIL, and 10% for LS. Each soil type was supplemented with carbon (0, 1, 2, and 3 mg glucose-C), nitrogen (0, 0.05, 0.1, and 0.15 mg NH_4NO_3-N), and phosphorus (0, 0.02, 0.04, and 0.06 mg KH_2PO_4-P). Bacterial counts were enumerated at 0, 3, 6, 12, 18, 24, 36, 48 and 72 hours on Sorbitol MacConkey Agar. The experiment was repeated 3 times.

Results: Without nutrient addition, CL supported the highest *E. coli* levels while SIL supported the lowest *E. coli* levels (P<0.05). Regardless of C concentration and soil type, C addition promoted *E. coli* growth and peaked within 24 hours (P<0.05) and then gradually declined and plateaued until 72 hours. The addition of 3 mg-C led to the highest *E. coli* growth by 18 hours (P<0.05), irrespective of the soil type. A slight increase in *E. coli* populations was found with an increase in N concentration in SIL and LS compared to the control after 72 hours (P<0.05). In SIL, 0.02 mg-P supported the highest *E. coli* populations at the end of 72 hours (P<0.05).

Significance: These results provide evidence that carbon and clay loam soil promote *E. coli* growth and survival. Additional studies should consider other nutrients and the soil microbiota to better understand pathogen behavior in soil.

P2-115 Minimum Concentrations of Pyrolyzed Paper and Walnut Hull Cyclone Biochar Required to Inactivate *E. coli* O157:H7 in Soil

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Introduction: Biochar has been shown to have antimicrobial properties in inactivating foodborne pathogens in soil. Previous studies have demonstrated the ability of high levels (ca. 10%) of biochar to inactivate *E. coli* O157:H7 (EC) in soil based on alkaline pH.

Purpose: A study was conducted to determine minimum concentrations of paper and walnut hull cyclone biochar required to inactivate EC in soil.

Methods: Soil was adjusted to 17.75% moisture and two types of slow-pyrolysis biochar ([1]Paper biochar, pyrolyzed at 700°C for 1 h and [2]walnut hull cyclone biochar, provided by All Power Labs, Berkeley, CA) were, respectively, added to soil at concentrations of 1.0, 1.5, 2.0, 2.5, 3.5, 4.5, 5.5, and 6.5% and mixed thoroughly. Samples were inoculated with 6.84 log CFU/g of a four-strain composite of nontoxigenic EC, mixed thoroughly, and stored at 21°C for up to 6 weeks. Experiments were performed in triplicate, samples were analyzed weekly, and EC populations determined.

Results: The soil-only control samples supported EC populations of between 6.86 and 6.01 log CFU/g between weeks 1 and 6. While all 1.0 and 1.5% concentrations, for each biochar type, resulted in a ≤1.6 log reduction at six weeks, all other treatments resulted in significant reductions of ≥2.0 log (p<0.05). Reductions of EC by paper biochar at the end of six weeks were 3.67 log at 2.0% concentration while 2.5-6.5% resulted in complete inactivation of EC by direct plating. In contrast, walnut biochar (2.0% concentration) effected only a 1.21 log reduction at six weeks, although 2.5-6.5% resulted in complete inactivation. Based on these results, concentrations of at least 2.5% paper or walnut hull cyclone biochar is required to achieve a >5.0 log reduction of EC in soil.

Significance: These results may provide guidance on the application of biochar added to crop soil to inactivate *E. coli* O157:H7.

P2-116 Survival of *Escherichia coli* TVS 353 as a *Salmonella* Surrogate When Using Composted Poultry Litter and Heat-Treated Poultry Pellets for Vidalia Onion Production

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❖ ***Developing Scientist Entrant***

Introduction: With recent *Salmonella* outbreaks associated with raw onion consumption, biological soil amendments of animal origin (BSAAO) are being examined as a potential route of contamination.

Purpose: The survival of *Escherichia coli* TVS 353, a surrogate for *Salmonella enterica*, was evaluated when using heat-treated poultry pellets and composted poultry litter to amend soils used in Vidalia onion production.

Methods: Treatment plots (40-in raised, 1 x 3 m, n=3) were inoculated with 1 L of rifampicin-resistant *E. coli* TVS 353 (9 log CFU/mL) and tilled with either composted poultry litter, heat-treated poultry pellets, or with no amendment added (unamended). At each time point throughout the production season, 30 g soil samples from each plot were quantified on tryptic soy agar with 80 µg/mL rifampicin. Most probable number (MPN) enumeration method was

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completed when *E. coli* levels went below 0.70 log CFU/g. Soil moisture content (%) and temperature (°C) were recorded. One-way ANOVA (p≤0.05) and Tukey's HSD were used to compare *E. coli* survival in plots.

Results: Initial *E. coli* levels in composted poultry litter, heat-treated poultry pellets, and unamended soils were 5.81±0.14 log CFU/g, 5.67±0.13 log CFU/g, and 5.69±0.14 log CFU/g, respectively. After 56 days, *E. coli* levels in composted poultry litter, heat-treated poultry pellets, and unamended soils were 3.40±0.21 log CFU/g, 3.34±0.19 log CFU/g, and 3.33±0.26 log CFU/g, respectively. *E. coli* levels decreased over 56 days in composted poultry litter, heat-treated poultry pellets, and unamended soils by 2.41±0.23 log CFU/g, 2.32±0.11 log CFU/g, and 2.37±0.32 log CFU/g, respectively. There were no significant (p<0.05) differences in *E. coli* levels based on amendment type on day 56.

Significance: *E. coli* persisted in soils used for Vidalia onion production. The majority of *E. coli* level decline was recorded between days 1-14 for all soil amendment types. Contaminated BSAAO could be a potential source of *Salmonella* contamination on onions.

P2-117 Survival of *Salmonella enterica* and *Escherichia coli* O157:H7 in Compost Amended Soils

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Introduction: There is a concern from the produce industry to use manure and compost for soil amendments, since the foodborne pathogens implicated in leafy green outbreaks most commonly originate from animals.

Purpose: The objective of this study was to assess the survival of *Salmonella enterica* and *Escherichia coli* O157:H7 over 65 days in 2 different composts amended with two different soils and determine any potential cross-contamination to plant tissue.

Methods: Loam or sandy loam soil, amended with poultry compost or a proprietary compost blend was inoculated with ca. 4 log CFU/g of either *S*. Newport or *E. coli* O157:H7 and mixed thoroughly. The amended soil samples were dispensed into planters, stored in a plant growth chamber and enumerated at days 0, 1, 3, 7, 10, 15, 25, 35, 45, 55 and 65 for surviving pathogens. Spinach was planted in the soils, and the plant samples were taken at days 25, 45 and 65 to determine the pathogen levels.

Results: For *S*. Newport, the population increased by 2-2.5 log CFU/g (n=2) at day 3, and maintained stable until day 45, then decreased by 0.9-1.1 log at day 65. The survival of *E. coli* O157:H7 showed different trends for the poultry compost and the proprietary compost amended soil. In poultry compost amended soil, *E.coli* O157:H7 population remained stable until day 10, then slowly decreased to 1.7 log at day 65, while in the proprietary compost amended soil, the population started to decrease at day 7 and reduced to below detection limit at day 65. No *S*. Newport or *E.coli* O157:H7 was detected in spinach plants at days 25, 45 and 65.

Significance: The findings can contribute to important data on how long pathogens can survive in various compost and soil types, and provide guidelines for safe application of composts in leafy green fields based on our findings.

P2-118 Persistence of Foodborne Pathogens in Biologically-Amended Soils and Produce on Integrated Crop-Livestock Farms on the Eastern Shore of Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Despite contamination risks associated with animal manures/composts, organic integrated crop-livestock farms (ICLFs) use biological soil amendments (BSAs) to improve soil health. The USDA National Organic Program (NOP) stipulates a withholding period of 120- and 90-days for high- and low-risk products, respectively. Previous studies have detected pathogens in soil and on produce 120 days post-BSA.

Purpose: This 10-month longitudinal study evaluated the effectiveness of the NOP withholding period by assessing microbial contamination of ICLF-soils and vegetables post-BSA.

Methods: Using standard methods, 410 manure/compost, soil, and corresponding, untreated produce samples were collected pre- and post-BSA and monthly until day180. All samples were analyzed for Aerobic plate count (APC), generic *Escherichia coli* (g*EC*), *Salmonella, Listeria monocytogenes* (*Lm*), Shiga toxin-producing *Escherichia coli* (STEC), and virulence factor (VF) genes using standard culture methods and PCR.

Results: Pre-BSA incorporation (Day-0A), 6.7% and 66.7% of manures/composts were positive for *Lm* and STEC/VF-genes, respectively. BSA enriched soils, and significantly (p>0.05) increased APC and g*EC* populations. All tested pathogens were detected in soils post-amendment (D0-B), with *Lm* and *Salmonella* prevalence increasing. Overall, *Lm*, *Salmonella*, and STEC/VF-genes were detected in 4.3%, 0.8%, and 24.0% of soil samples, respectively, with increased chance of contamination when total coliforms were 4-5 log CFU/g. Regarding the withholding period, one soil sample was positive for *Lm* on Day-90 and *Salmonella* on Day-120, while STEC/VF-genes were detected on both days. For produce samples, 3.5%, 0.7%, and 14.2% were positive for *Lm*, *Salmonella*, and STEC/VF-genes. For produce, *Lm* was isolated on Day-90 and Day-180, *Salmonella* on Day-180, and STEC/VF-genes were detected from Day-90 onward.

Significance: Fecal and pathogenic contamination of BSAs, and corresponding soils and produce emphasize the risks associated with BSAs. Findings from this study highlight the persistence of pathogens relative to the NOP withholding period, providing important evidence regarding application intervals.

P2-119 Detection and Prevalence of Major Foodborne Pathogens in Integrated Crop-Livestock Farms and Post-Harvest Products on the Eastern Shore of Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Concerns remain about the extent of zoonotic pathogen contamination risk to fresh produce from Integrated Crop-Livestock Farms (ICLF) due to proximity of farm animals to crop fields and use of manure-based soil amendments.

Purpose: To determine pathogen contamination from soil, animal-rearing areas, produce, and water within ICLFs on Maryland's Eastern Shore and compare results to those from crop-only farm (COFs), farmers-market (FMs), and retail-store (RTs) samples.

Methods: Monthly samples were collected from soil and animal-rearing areas; untreated produce was acquired at FMs and RTs. Water was collected summer and fall. A total of 1,782 samples were tested for aerobic plate count (APC), generic *Escherichia coli* (g*EC*), *Salmonella*, *Listeria monocytogenes* (*Lm*), Shiga toxin-producing *Escherichia coli* (STEC) and virulence factors (VFs) using standard culture methods and PCR.

Results: ICLF-soils had increased contamination risk with 16.2%, 2.1%., 2.8%, and 21.1% samples positive for g*EC*, *Salmonella*, *Lm*, and STEC/VF-genes, respectively, compared to 5.3%, 1.3%, 0.0%, and 20.0% for COF-soils. Increased risk was likely associated with proximity of on-farm animals and use of manures/composts. When combined, animal pen and manures/composts had the highest percentages of detectable fecal and pathogenic bacteria among all samples. Similarly, g*EC*, *Lm*, and STEC/VF-genes were detected more frequently from ICLF-produce than from COF-produce. The 13.9% STEC/VF-gene positive ICLF-produce was significantly greater than (p<0.05) 5.3% positive COF-produce. *Salmonella* wasn't isolated from ICLF, COF, or RT-produce, but was

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positive in 0.4% FM-produce. Both ICLF and COF-produce had significantly ($P<0.05$) greater incidences of STEC/VF-genes compared to FM and RT-produce. Bareilly, Newport, and Thompson were the highest *Salmonella* serovars detected, while O103, O157, *stx*2, and *ehx*A represented the top STEC/VF-genes.

Significance: Pathogen prevalence in ICLFs, COFs, and produce from market/retail environments indicates fresh produce production risks. Research is needed to highlight pathogen survival/transfer and efficient risk-mitigation strategies in these unique environments.

P2-120 Effect of Treated or Untreated Bsaao Application on Microbial Food Safety Risk on Carrots Irrigated with Contaminated or Chlorinated Water

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❖ ***Developing Scientist Entrant***

Introduction: The application of BSAAO's can improve plant growth and development, however, it can also serve as a risk of contamination for root crops.

Purpose: This study examined the effectiveness of different levels of chlorination for irrigation water and BSAAO application to minimize microbial risk of carrots.

Methods: A production plot of 360 ft^2 with 45 elevated beds (30 ft long) with BSAAO applications (no BSAA, compost, and raw manure tilled or no-till) and irrigation (non-chlorinated, 3ppm and 50ppm residual chlorine) were planted with Scarlet Nantes carrots, for split-plot design. Soil samples from each treatment were collected for the first five weeks to examine naturally present *Escherichia coli*/coliforms, carrots were harvested at weeks 9 and 10. Plots were irrigated with respective chlorination levels once daily throughout the study, with 27.5 gal of water being applied to each column of beds.

Results: E. coli levels in soil were significantly higher in plots with raw manure regardless of irrigation treatments, with average populations ranging from 1.02 to 3.13 log CFU/g. *E. coli* on the surface of carrots harvested from plots irrigated with 50ppm was higher from plots with no-till raw manure (2.53±0.55 log CFU/g) than all other BSAAO treatments (ranging from 0.52 to 1.53 log CFU/g). Carrots harvested from plots irrigated with 3ppm has significantly higher levels of *E. coli* from plots with tilled raw manure or no-till compost (2.01±1.06 and 1.99±0.42 log CFU/cm^2 respectively) than plots with no BSAAO applied (1.02±0.86 log CFU/cm^2). Carrots irrigated with 50ppm (46.02±21.27 g) were larger than those irrigated with non-chlorinated water (35.86±16.26 g). *E. coli* O157:H7 was consistently detected in plots with raw manure.

Significance: The level of residual chlorine in irrigation water has variable effect on *E. coli* and total coliforms levels on carrots and were significantly affected by the presence or absence of organic material in soil.

P2-121 Stable Isotope Probing is a Valuable Tool for Studying Microbial Community Interactions of *Listeria monocytogenes* in Cantaloupe Soil Mesocosms

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Introduction: Listeria monocytogenes (LM) has been implicated in several fresh produce outbreaks linked to cantaloupe production. Despite the recurrence of *LM* outbreaks and significant efforts from the industry to reduce them, little is known about the interactions of LM with farm soil microbial communities (MC).

Purpose: To determine the MC interactions between rhizosphere microorganisms and *Listeria* species (LS) in cantaloupe mesocosms using stable isotope probing (SIP).

Methods: ^{13}C and ^{12}C growth curves were performed for LS and the concentration of incorporated ^{13}C was determined at stationary phase. Cantaloupe (cv Athena) mesocosms were inoculated with ^{13}C or ^{12}C labeled *LS* or phosphate buffer. Soil samples were collected from 4-separate locations inside the mesocosm at day 0, 1, 4, 8, 15, 24, and 36 for DNA extraction and bacterial enumeration. ^{13}C and ^{12}C soil DNA was separated using SIP for MC analysis. Soil and DNA ^{13}C concentrations were determined by the UC-Davis SIP-Facility.

Results: Listeria innocua (LI) and *LM* growth patterns were identical in ^{13}C and ^{12}C media. *LI* incorporated more than 98 atom% of ^{13}C after 24 or 30h of incubation. No significant difference in ^{13}C s concentrations was determined between the 2-time points (P=0.716). Isotopic signature (IS) had no effect on *LS* die-off rates (DOR) (P=0.481). DOR was significantly impacted by strain and sampling-day (P<0.0001). Linear DOR of ^{13}C and ^{12}C labeled *LS* (0.190 +/- 0.03 and 0.194 +/- 0.03 cfu/day) were not significantly at 36 days post-inoculation (P-I), with IS and strain-type impacting survival/persistence of *LS*. ^{13}C soil IS revealed no differences between ^{13}C and ^{12}C treated mesocosms (P=0.720). However, significant differences in ^{13}C IS were found between ^{13}C and ^{12}C enriched LS (P≤0.001; mean comparison -4.7).

Significance: LS persisted in soil for over 30 days P-I irrespective of IS and with identical linear DOR. SIP had no negative impact on growth/persistence of LS.

P2-122 Presence and Antibiotic Resistance of *Acinetobacter spp., Salmonella spp., Pseudomonas* spp. and *Escherichia coli* in the Agricultural Environment of the Cantaloupe Melon

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❖ ***Developing Scientist Entrant***

Introduction: The presence of antimicrobial-resistant bacteria is increasing in the agricultural environment. Contamination of fruits and vegetables can occur during the production chain, and little information is available in the melon producing environment.

Purpose: To detect, isolate and identify *Acinetobacter* spp., *Salmonella* spp, *Pseudomonas* spp. and pathotypes of *Escherichia coli* (EPEC, EHEC), from soil and fruit samples from cantaloupe melon farms in México and determine their antibiotic resistance and the presence of virulence genes.

Methods: A total of 48 agricultural samples were collected in Coahuila Mexico. These were composed of 12 composite samples of melon (each composed of three melon rinsates) and four composite samples of soil (composed of three soil samples each). Detection of *Acinetobacter* spp., *Salmonella* spp., *Pseudomonas* spp. and *E. coli* pathotypes was conducted using conventional methods. Ten typical colonies were selected from each agar according to their phenotypic characteristic and confirmed by PCR. These isolates were screened for genes related to toxins, adhesins and invasion virulence factors. Each isolate was analyzed for antibiotic resistance to ampicillin (AMP), erythromycin (E), tetracycline (TE), cefotaxime (FOX), sulfamethoxazole trimethoprim (SXT) and amoxicillin clavulanic acid (AMC) in Muller Hinton agar.

Results: A total of 366 presumptive bacterial isolates were obtained, and 116 exhibited one or more of the virulence genes analyzed. Thirty-nine isolates were identified as *Acinetobacter* spp (16s rRNA $^{+}$), eight as *Salmonella* spp (*InvA* $^{+}$), 66 as *Pseudomonas* spp (*OprL* $^{+}$ and *OprI* $^{+}$) and three as *E. coli* (*eae*$^{+}$). All isolates were resistant to AMP and resistant to at least three of the antibiotics tested. Most of isolates were susceptible to TE, except *Salmonella* in which all isolates were resistant.

Significance: The analyzed agricultural environment of melon contains pathogenic bacteria that are multi-resistant to antibiotics, and pose a risk to public health. Strategies need to be applied to minimize the impact.

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P2-123 Risk Factors Associated with the Presence of Generic *E. coli* in Fresh Produce Fields with Crop-Livestock Integration in California and Minnesota

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❖ ***Developing Scientist Entrant***

Introduction: Livestock manure in cover-cropped fields may introduce foodborne pathogens to fresh produce while integrated crop-livestock farm (ICLF) grazing fertilizes soils. Multiple factors can influence survival of foodborne pathogens in manure-amended soils.

Purpose: To identify risk factors for the presence/persistence of generic *E.coli* (gEc) in ICLF soils in CA and MN.

Methods: A randomized complete block experiment was conducted on certified organic fields in CA and MN (2021-2022), with three treatments (winter cover-crop, grazed with sheep (WG), tilled (WT), and fallow (WF)). For assessing presence/persistence of gEc, as a surrogate for foodborne pathogens in soil, 36 soil samples were collected on 0-, 7-, 30-, 60-, 90-, and 120-days post-grazing (DPG). Meteorological data including precipitation, solar radiation, air and soil temperatures, wind speed and relative humidity were retrieved from the closest weather stations to the CA and MN fields. Mixed effect logistic regression with subplot as a random effect was used to assess risk factors and treatment effects on the presence of gEc.

Results: Among 936 soil samples collected, 343 (36.6%) were gEc positive. In the final mixed effect multivariable logistic regression model with sampling days (DPG) and treatment, the odds of soil contamination with gEc showed significant positive association with the cumulative amount of rainfall during the 29 days preceding sample collection (OR=1.05, p=0.005), and negative association with soil average temperature during the 2 days preceding sample collection (OR=0.95, p=0.023). Significant increases in gEc prevalence were observed at 30-DPG (OR=18.03, p<0.001) and 60-DPG (OR=6.75, p=0.006) in WG, but no significant difference compared to D0 was observed after 90-DPG in all treatments.

Significance: The study provided potential meteorological risk factors affecting gEc presence in ICLF systems. Results indicate application of USDA's National Organic Program 90-120 day wait-period between manuring and harvesting in ICLFs take into account recent meteorological events.

P2-124 Analyzing Predominant Serotypes and Antibiotic Resistance Profiles of *Salmonella enterica* Isolated from Integrated Farms in the MD-D.C. Area

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❖ ***Developing Scientist Entrant***

Introduction: Salmonella enterica (SE) remains a ubiquitous pathogen in the food producing systems. Food products which are grown in integrated and backyard farms are also involved in sporadic and/or limited outbreaks of various enteric pathogens including SE. Further, the nationwide increase in detection of antibiotic resistant SE isolates has also warrants further monitoring these trends, along with the prevalence of this pathogen in integrated/backyard farm environment and their products.

Purpose: Evaluate the predominance of specific serotypes of SE and their antibiotic resistance pattern within integrated/backyard farms in the MD-DC area.

Methods: Isolated SE from farms that engage in pasture and integrated farming practices were serotyped using multiplex PCR. Resistance to 13 clinically relevant antibiotics was evaluated through an antibiogram assay performed by inoculating each isolate in Mullen-Hinton agar individually supplemented with pre-determined standard CLSI concentrations. Additional qPCR showed relative expression of multidrug resistance genes in highly resistant isolates.

Results: A total of 144 isolates from integrated/backyard farming environments and their products, were serotyped, revealing major identifiable serotypes to be Typhimurium (11.11%), Hadar (7.64%), Enteritidis (5.55%), Gallinarum (3.47%), Pullorum (2.77%), Agona (2.77%), Heidelberg (2.08%), Newport (1.38%), Westhampton (1.15%) and other (66.66%). Antibiogram showed most isolates to be resistant to ampicillin (88.69%), amoxicillin (80.87%), cephradine (75.65%), streptomycin (71.30%) and ceftriaxone (62.61%), followed by trimethoprim/sulfamethoxazole (43.48%), tetracycline (41.74%), oxytetracycline (41.74%), azithromycin (39.13%), chloramphenicol (26.95%) and ciprofloxacin (20.87%), with most being susceptible to kanamycin (92.17%) and gentamycin (99.13%). Further, relative expression of genes associated with multi-drug resistance in an isolate resistant to 11 antibiotics showed significant (p<0.05) upregulation of *sdiA* and *mdfA* by 0.71 and 0.81 log, respectively, compared to control, while being 3.14 and 4.01 log when compared to an isolate resistant to only 3 antibiotics.

Significance: Monitoring major serotypes and their antibiotic resistance patterns remains an important aspect of food safety, especially products grown in integrated/backyard farms.

P2-125 Dominance and Antibiotic Sensitivity of *Campylobacter* at Mixed Farms in Maryland-Washington DC Area

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Introduction: *Campylobacter* is responsible for a huge number of foodborne diarrheal diseases in the US. This enteric pathogen transmits to food products through different components of the farm including soil, water, and livestock, and ultimately lead to foodborne illnesses in human. Mixed or integrated farms, in which farmers grow crop and livestock side by side and recycle the waste materials, poses more risk in cross-contaminating their products.

Purpose: This study is aimed to determine the dominance of *Campylobacter* at various components of mixed farms in the Maryland-Washington DC area and their sensitivity against commonly clinically used antibiotics.

Methods: Different categories of samples (n=3,223), including soil, water, grass, feces, compost, feed, bedding, and produce (both pre-and post-harvest), were collected from mixed farms in the Maryland-Washington DC area. Collected samples were enriched and plated on selective media for presumptive isolation of *Campylobacter*. Presumptive isolates were then confirmed by PCR for 16sRNA. The resistance pattern of the confirm *Campylobacter* isolates against common clinically used antibiotics was determined following the method recommended by Clinical and Laboratory Standard Institute.

Results: A total of 14.65% (472/3,223) samples were found contaminated with *Campylobacter* and collected produce samples 10.58% (341/1,720) were found highly contaminated. Among these *Campylobacter* isolates, 23.1%, 24.36% and 52.54% were *Campylobacter jejuni*, *C. coli* and other *Campylobacter* spp., respectively. All the isolates were resistant to at least one of the antibiotics tested and streptomycin, and gentamycin were found more effective, and tetracycline, ampicillin, and ciprofloxacin were less effective against these isolates.

Significance: The presence of *Campylobacter* in the farm environmental and produce samples showed the strong possibility with the chances of cross-contamination of the mixed farm produce but most of the *Campylobacter* isolates were treatable with common clinically used antibiotics.

Poster

P2-126 Sanitizer Type and Contact Time Influence *Salmonella* Reductions in Preharvest Agricultural Water Used on Virginia Farms

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❖ ***Developing Scientist Entrant***

Introduction: Contaminated preharvest agricultural water can transmit foodborne pathogens to crops. No Environmental Protection Agency chemical treatments for preharvest agricultural water are currently labeled to reduce foodborne pathogens.

Purpose: Examine the efficacy of peracetic acid- (PAA) and chlorine- (CL) based sanitizers against *Salmonella* in a Virginia pond and river used for crop irrigation.

Methods: Surface water was collected at three time-points during the growing season (May, July, September). Water samples (100ml) were inoculated with either a 7-strain EPA/FDA-prescribed cocktail or a 5-strain clinical *Salmonella* outbreak cocktail. Experiments were conducted in triplicate for each strain-set, sanitizer, water-type, time-point, temperature (12 or 32°C), concentration [low (PAA:6, CL:2-4ppm) or high (PAA:10, CL:10-12ppm)], and contact time (1, 5, or 10min) combination (total combinations=288, total samples=864). *Salmonella* were enumerated by spread-plating after each treatment combination and log reductions calculated. A log-linear model was used to characterize how each treatment combination influenced *Salmonella* reductions.

Results: *Salmonella* reductions ranged from 0.0±0.1 to 5.6±1.3 log CFU/100ml for PAA, with 50% (72/144) of PAA treatments achieving ≥three-log reduction. *Salmonella* reductions ranged from 2.1±0.2 to 7.0±0.2 log CFU/100lL for CL, with 97.2% (140/144) of CL treatments achieving ≥ three-log reduction. Physicochemical parameters significantly varied by untreated water-type; however, *Salmonella* reductions did not (*P*=0.14), possibly due to variable sanitizer quantities needed to achieve the target residual concentration in the treated water. Significant differences (*P*<0.05) in *Salmonella* reductions were observed for treatment combinations, with sanitizer (CL>PAA) and contact time (10>5>1min) having the greatest effect. While the strain-set used did have a significant impact on *Salmonella* reductions, these reductions were less than the effects of sanitizer type, contact time, or sanitizer concentration.

Significance: Most treatment combinations (73.6% or 212/288) achieved a > three-log CFU/100ml using the FDA-EPA protocol. Awareness and monitoring of water quality parameters (e.g., turbidity) are essential for ensuring adequate dosing for effective treatment of preharvest agricultural water.

P2-127 Not All Ponds are Created Equal: Factors Associated with *Salmonella* Contamination Varies by Pond and Detection Method

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❖ ***Developing Scientist Entrant***

Introduction: Agricultural water needs to be of adequate quality to limit potential produce contamination. Understanding factors that impact microbial water quality are important to managing agricultural water.

Purpose: This study sought to evaluate factors associated with *Salmonella* contamination in Virginia ponds.

Methods: Grab samples were collected twice per week from nine ponds (30 sites, 600 samples total) within a 187ha area. Total coliform and *Escherichia coli* (FIB) levels were enumerated using IDEXX Quanti-Trays. *Salmonella* was detected in 250mL water samples by both molecular (*invA* gene) and culture-based methods. Data on water quality and environmental factors were also collected for each sample and site. Mixed-effects Bayesian regression was used to quantify associations between *Salmonella* and each factor; models were run using data from all ponds combined, and each individual pond, by detection method.

Results: *Salmonella* was detected in 30.5% (183/600) and 12.8% (77/600) of samples for molecular and culture-methods, respectively. The odds of *Salmonella* isolation differed between the nine ponds [Odds Ratio (OR): 2.67-16.20] regardless of isolation methods. When data were aggregated for all nine ponds, increased FIB levels resulted in increased odds of *Salmonella* (OR: 1.30-1.70). Rain while sampling (OR: 3.62-5.09), air temperature (OR: 0.78-0.85), and solar radiation (OR: 0.24-0.53) were all strongly associated with *Salmonella* detection for both isolation methods; however, four additional factors were determined to be non-negligibly negatively associated with *Salmonella* isolation via culture-methods. For individual ponds, the presence, direction, and strength of association between each factor and *Salmonella* detection varied, with some associations only being observed in certain ponds.

Significance: Findings show that methodological differences and study scale confound pathogen isolation in surface water. Additionally, since models built using aggregated data masked associations at the individual pond levels, results suggest management of water needs to be individualized (i.e., assess hazards/risks by individual pond), even if waterways are on the same farm.

P2-128 Metabolic Diversity of *Salmonella* Newport Isolated from East Coast Agricultural Environments

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Introduction: There is a need to better understand the ecology of *Salmonella* interactions within the environment and the mechanisms used to persist within soil, sediment, surface waters, and agricultural commodities. Using *S*. Newport as a model, we are able to gain a better understanding of the traits that allow *Salmonella* to survive within these environments, leading to a more comprehensive view of how the environment is impacting food safety.

Purpose: To identify potential adaptive differences in *S.* Newport isolates from three separate lineages using phenotypic microarray analysis.

Methods: Twenty *Salmonella* strains were selected for this analysis representing Newport isolates (n=16) of various genotypic linages and source (environmental, clinical, or historical) and non-Newport isolates (n=4) associated with outbreaks linked to fresh produce. Bacterial strains were evaluated using phenotypic microarray assays in biological triplicate at 30°C for 48 hours. Metabolic substrates evaluated were carbon (PM1, PM2A), nitrogen (PM3B), phosphorus/sulfur (PM4A), nutrient supplements (PM5), osmolytes (PM9) and pH (PM10). Area-under-the-curve was calculated using Data Analysis 1.7 and significant differences between strains were identified using a two-sided t-test and the Benjamini-Hochberg procedure.

Results: The majority of differences in substrate utilization between strains were identified in carbon source, with D-Saccharic acid (n=19) and m-Tartaric acid (n=10) metabolization being most significantly different between strains, *P*<0.005. Isolates which were obtained directly from environmental sources had the most statistically significant metabolization patterns when compared to non-environmental isolates. No significant differences were found between the strains tested in the presence of different osmolytes or varying pH levels.

Significance: Differences in phenotypic array profiles shed light into the carbon, nitrogen, phosphorous, and sulfur substrates utilized by the different Newport lineages from different environments, which will aid in new strategy development for *Salmonella* pre-harvest prevention.

Poster

P2-129 Cleanliness of Over-the-Row Blueberry Machine Harvesters Washed and Sanitized with Various Approaches

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❖ ***Developing Scientist Entrant***

Introduction: Contamination of fresh blueberries via their contact with equipment surface has been recognized as an important food safety issue. The surface of over-the-row (OTR) machine harvesters could be a potential source of contamination during harvest. Blueberry growers currently use different approaches to clean/sanitize machine harvesters.

Purpose: This study examined the hygiene condition of the surface of OTR machine harvesters cleaned/sanitized with different approaches.

Methods: Four or six machine harvesters in Georgia and Oregon respectively were each sampled twice on two different harvest days in the summer of 2022. Nine sites (upper and lower side walls, upper and lower beating bars, catcher plates, horizontal and vertical conveyors, lugs, and filling flap) on the top loaders (n=9) and seven sites (excluding vertical conveyor and filling flap) on the bottom loaders (n=2) were sampled before and after cleaning/sanitation. Populations of total aerobes (TA), total yeast and mold (YM), and total coliforms (TC), as well as the presence of fecal coliforms (FC), and enterococci (EC) in the swab samples were determined. Data collected were analyzed using the Split-Plot ANOVA of SAS.

Results: In Georgia, cleaned/sanitized surfaces had significantly (P<0.05) lower TA and YM counts than the uncleaned surfaces, while no difference in TC counts was observed. The vertical and horizontal conveyors and the catcher plates had significantly higher TA, YM, and TC counts than other sampled sites. Similar findings were noticed with samples collected in Oregon. FC and EC were detected in 7.8% or 14.1% of the Georgia samples and 5.6% or 10.2% of the Oregon samples.

Significance: The type and concentration of sanitizers and the frequency of cleaning/sanitation treatments all had an impact on the hygiene status of berry-contact surface of OTR machine harvesters. The cleaning/sanitation approaches used by participating growers are largely working, but efforts could be made to improve the hygiene conditions of machine harvesters.

P2-130 Aggregative Soil Sampling Using Pre-Hydrated Bootie and Drag Swabs Shows Similar Indicator Bacteria Recovery Ability in Comparison to Grab Soil Sampling from Commercial Romaine Fields

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❖ ***Developing Scientist Entrant***

Introduction: Soil represents an important contributor to pre-harvest food safety risk. However, typical grab soil sampling may be under representative due to sampling location and mass limitations. Aggregative soil sampling by testing bootie and drag swabs might be more representative.

Purpose: To compare bootie and drag sampling methods with composite grab soil sampling.

Methods: Sampling was performed in six beds of commercial romaine lettuce fields in Salinas, California. Three sampling methods were used to take soil samples: booties (wearing sterilized, perforated, and hydrated bootie covers), drags (dragging hydrated gauze), and grabs (grabbing composite soil samples using shovels). Twenty samples were taken for each method; Bootie and drag swabs each covering approx. 333 ft paired with 25 grams of well-mixed grab samples. Samples were processed to enumerate generic *Escherichia coli*, total coliforms, and aerobic plate counts. ANOVA and Tukey's test were used to compare means.

Results: There was no generic *E. coli* detected in any sample (LOD=1CFU/ml). Total coliforms recovery on booties, drags, and grabs were 2.50 ± 0.89, 2.91 ± 1.02, and 1.94 ± 0.48 log CFU/g, respectively. Grabs showed a lower (P=0.001) mean than drags, whereas booties showed no significant difference from either grabs (P=0.088) or drags (P=0.27). Aerobic plate counts recovery on booties, drags, and grabs were 7.03 ± 0.30, 7.16 ± 0.11, 6.98 ± 0.11 log CFU/g, respectively. The means of booties (P=0.024) and grabs (P<0.001) were lower than drags. There was no significant difference (P=0.44) between grabs and booties.

Significance: This data suggest that bootie and drag swabs performs similar to, at least not worse, than composite grab for indicator organism testing in soil from common produce fields. Future work is planned to determine if this is a more powerful method for pathogen detection in inoculated fields.

P2-131 Aggregative Sampling using Prehydrated Cloths Performs No Worse Than Tissue Sampling in Recovering Quality and Safety Indicators from Commercial Romaine Lettuce Fields

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❖ ***Developing Scientist Entrant***

Introduction: A previous pilot study demonstrated that manual aggregative sampling using dry cloths performs no worse than composite produce sampling in the recovery of quality indicators but underperforms recovering safety indicators

Purpose: This study aims to improve the aggregative sampling technique by testing pressure and hydration as factors affecting the recovery of quality and safety indicators to justify future, more expensive, work on pathogen recovery.

Methods: Sampling was performed in 400-m long beds of romaine lettuce in commercial fields in Salinas, California. Aggregative sampling consisted of collecting: (i) Dry and pre-hydrated cloths (testing for hydration), (ii) collected by hand or using Manual Sampling Device (MSD) (testing for pressure) (n=31). Composite produce samples were collected for comparison (n=21, 375 g/sample). Samples were tested for quality metrics: aerobic plate counts (APC), and safety indicators: coliform counts and generic *E. coli.* Mean counts were analyzed using ANOVA and generic *E. coli* detection using Chi-square test.

Results: Pre-hydrated cloth samples showed significantly higher (p<0.001) mean APC and coliform counts, 6.67±0.94 log(CFU/g) and 6.48±0.94 log(CFU/g) respectively, compared to dry cloths that showed APC of 5.08±0.3 log(CFU/g) and coliform counts of 2.03±1.29 log(CFU/g). Pressure showed no significant effect (p=0.13) on bacterial recovery. Pre-hydrated cloths attached to the manual sampling device was considered the best sampling technique. These samples showed significantly higher (p<0.001) mean APC and coliform counts, 6.67±0.94 log(CFU/g) and 6.48±0.94 log(CFU/g) respectively, compared to paired composite samples that showed 5.28±0.36 log(CFU/g) and 4.91±0.8 log(CFU/g), respectively. Generic *E. coli* was found in 9/13 produce samples, while 6/15 improved aggregative samples, this was insignificantly different (p=0.24).

Significance: This study suggests that using pre-hydrated cloths in aggregative sampling improves the recovery of APC and coliforms and performs no worse in the recovery of generic *E. coli* when compared to current composite produce grab sampling. It justifies future work on pathogen detection.

P2-132 Evaluation of Peptones for Optimal Recovery of Airborne Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: Dispersal of bacteria in the air can cause the spread of potential pathogens. Collection media used to collect samples of aerosolized bacteria could affect capture and recovery.

Purpose: To evaluate if different peptone solutions will affect the recovery of aerosolized bacteria.

Methods: 10 different 0.1% peptone solutions (Starter Pak No.2 & 3, Gibco™, CAT #:215367, 215368) and sterile deionized water (SDW) were prepared as collection media from A solution (~8 log CFU/ml) of *Escherichia coli* K12, with ampicillin resistance and GFP expression, was placed into an AFFROG mini diffuser. This diffuser was placed inside of a VIVOSUN grow tent (48x24x60 inches) 1 foot from the left wall and at a height of roughly 1 foot. Glass impingers filled with 100 ml of collection media were connected to air pumps (Gilian BDX-II, Sensidyne) and placed in the tent 1 foot from the right wall. The diffuser was turned on for 15 minutes (~0.5 ml/minute). After 15 minutes, the diffuser was turned off, and the impinger's air pumps (~2.5 ml/min) were turned on for 1 hour of collection. After 1 hour, samples were enumerated.

Results: There was no significant difference between *E. coli* recovery for the 10 tested peptones (p>0.05). Bacto™ Malt Extract, Bacto™ TC Yeastolate, Bacto™ Yeast Extract, Gibco™ Yeast Extract, and Fisher BioReagents™ Peptone showed significantly higher recovery than SDW (p<0.05). Bacto™ Malt Extract exhibited the highest average recovery (3.44±.23 log CFU/ml). Of the peptones, Bacto™ Proteose Peptone No.2 (2.65±1.9 log CFU/ml) and No.3 (2.58±.33 log CFU/ml) showed the lowest recovery. Porcine based peptones showed the lowest average recovery amongst the peptones (2.62±.24 log CFU/ml) and was the only source type to not show significantly greater average recovery than SDW (p>0.05).

Significance: Peptone sources for the preparation of 0.1% peptone buffer could influence the recovery of aerosolized *E. coli*.

P2-133 Evaluation of Indicators of Microbiological Air Quality in Peach Orchards

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❖ ***Developing Scientist Entrant***

Introduction: Dust and aerosols have been shown to disperse bacteria in pre-harvest environments. Bile, endotoxins, *Pseudomonas* spp. and heterotrophic plate counts of air samples from peach orchards were correlated with presence of coliforms in air.

Purpose: To evaluate the presence of biomarkers as a potential indicator of microbial air quality on peach farms in comparison to other microbial counts and environmental data.

Methods: Air samples were collected at 9 different peach orchards in southern Georgia. The samples were collected using glass impingers connected to air pumps (Gilian BDX-II, Sensidyne) for 1 hour (~2.5 ml/min). Leaf and soil samples were collected along with environmental data (Particle count, Air temperature, Relative humidity, Dew point, & Wet bulb).Air samples were analyzed for heterotrophic, coliform, and *Pseudomonas* presence using MPN methods. For HPC and coliforms, the IDEXX Quanti-Tray 2000 system was used. For *Pseudomonas*, a miniaturized, Resazurin based 96-well plate MPN was used. Air samples were analyzed for bile acid and endotoxin levels. All samples were collected on 3 separate days throughout the 2022 peach season.

Results: Across the samples, there was a moderate correlation between average HPC and *Pseudomonas* levels in both air (r=0.41) and leaf samples (r=0.58). There was moderate correlation between HPC and coliforms in the air samples (r=.43). There were low levels of both bile and endotoxin found in 9 different air samples. For coliforms in the air samples, there was a slight negative correlation (r=-.28) with endotoxin levels, a slight negative correlation (r=-.29, -.22, -.23) with the particulate matter (0.3, 2.5, 10 µm), and a moderate correlation (r=.62) with bile levels. There was no significant difference between the microbial counts for air, leaf, or soil samples between the 9 orchards (*P*>0.05).

Significance: Biomarkers such as bile and endotoxins could potentially serve as an indicator of air quality in a pre-harvest peach orchard environment.

P2-134 Survival of *Salmonella enterica* and *Listeria monocytogenes* in Hydroponic Pond Water as Affected by Water Microbiota

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Introduction: Deep Water Culture (DWC) is a popular controlled environment agriculture (CEA) system of hydroponic leafy green production. However, the microbiological quality and the risks of DWC pond water supporting the growth and survival of foodborne pathogens are largely unknown.

Purpose: To determine the microbial load of DWC water and the effect of water microorganisms on the survival of *Salmonella enterica* and *Listeria monocytogenes*.

Methods: Water obtained from commercial DWC ponds at 2 hydroponics facilities were filtered through 300, 1, and 0.2 µm membranes to selectively remove debris, eukaryotic microorganisms and protozoa, and bacteria, respectively. Filtered water was tested for aerobic bacteria (APC), and yeast and mold (YM), and subsequently inoculated with *S. enterica* and *L. monocytogenes.* The survival of *S. enterica* and *L. monocytogenes* was monitored for 7 days at 23 °C and 75 rpm.

Results: APC and YM in filtered DWC water were undetectable (in 0.2 µm filtrates), 3.2 to 5.8 and undetectable (1 µm), and 3.3 to 6.7 and 2.1to 3.8 (300 µm) log CFU/ml, respectively, while *S. enterica* and *L. monocytogenes* were not detected. After inoculation, *S. enterica* population in 0.2 µm filtrates increased by approx. 2 logs by day 3 and was stably maintained by day 7. *S. enterica* in 1 and 300 µm filtrates declined at varying rates higher than that in sterile distilled water control, with that in 300 µm filtrates to a mostly undetectable level by day 3. *L. monocytogenes* population change followed similar trends as for *S. enterica*, except that it did not grow even in 0.2 µm filtrates.

Significance: This study suggests DWC water microbiota play a critical role in determining the growth and survival of foodborne pathogens in hydroponic leafy green production systems. Future work will focus on the identification and characterization of such pathogen antagonist microbiota from various CEA production systems.

P2-135 Survival and Persistence of *Listeria* and *Escherichia coli* and Changes in Physicochemical Parameters in Aquaponics Systems during Lettuce Production

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Introduction: Aquaponic (AP) food production systems integrate aquaculture and hydroponics in soilless controlled environments while conserving space and natural resources (soil, water, air). Increasing global demand for high quality nutritious, safe food continues increasing exponentially. Information specific to AP is needed to validate operational and handling practices for AP food safety.

Purpose: Evaluate survival of inoculated *Listeria innocua* and nonpathogenic *Escherichia coli* and changes in AP physicochemical parameters.

Poster

Methods: A four-week bench-scale AP experiment (n=12) with four goldfish (*Carassius auratus*)/aerated 37L tank, a three-step biofilter, and four lettuce plants (*Lactuca sativa var. Truchas*)/16L deep water hydroponic tank was conducted. Treatments were high and low-dose (6 and 2 log CFU/ml, respectively) of *Listeria innocua* 2066-^{Er} or *E. coli*-^{Rifr} TVS 354, and uninoculated controls. *Listeria innocua*-2066-^{Er} and *E. coli*-^{Rifr} populations, mesophilic counts (APC), and physicochemical parameters (pH, temperature, dissolved oxygen, turbidity, ammonia, nitrite, and nitrate) were analyzed in plant tank water and biofilters until plant harvest. Levels of *Listeria innocua* 2066-^{Er} and *E. coli*-^{Rifr} and APC (Petrifilm®) from lettuce shoots, roots, and rockwool were determined at harvest.

Results: *Listeria* and *E. coli* populations declined significantly in both high and low-dose treatments within 24 h post-inoculation and were undetectable at day 14 and day 12 respectively ($P<0.05$). *Listeria* and *E. coli* were detected in biofilters until week 4. At harvest, *Listeria* and *E. coli* were recovered from lettuce roots, and rockwool, but not from plant leaves and 100ml plant tank water grab samples. Lower leaves pre-harvest had a significantly higher APC (5.1 to 6.4 log CFU/ml) relative to harvested lettuce upper leaves (2.8 and 4.2 log CFU/ml). Plant tank water pH had a significant effect ($P<0.05$) on *Listeria* and *E. coli* survival.

Significance: These results provide insights into the survival dynamics and sites of *E. coli* and *Listeria* in AP and associated physicochemical conditions.

P2-136 Evaluation of Calcium Chloride and Peracetic Acid to Inactivate *E. coli* and *Salmonella* in Irrigation Water in Maryland

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❖ ***Developing Scientist Entrant***

Introduction: Addition of antimicrobials to water used for irrigation of fresh produce can significantly reduce microbial food safety risk. However, there is a need to validate the efficacy of these compounds in irrigation water using the microbial strains and methods recently recommended by the Environmental protection Agency (EPA).

Purpose: To evaluate the antimicrobial performance of calcium hypochlorite ($Ca(ClO)_2$) and peracetic acid (PAA) in ground and surface agricultural production water in Maryland using the EPA recommended method (No. 94151PA7).

Methods: Ground and surface water were collected in Maryland and stored at 4°C. One ml of EPA Agricultural Water Panel *Escherichia coli* or *Salmonella* cocktail was inoculated in 98 ml of irrigation water in three flasks and equilibrated at 12 or 32°C. Each trial was initiated by transferring 1 ml of $Ca(ClO)_2$ or PAA solution into the flask to achieve the targeted levels (2 to 4 and 10 to 12 ppm for $Ca(ClO)_2$; 6 and 10 ppm for PAA), followed by sample mixing for 15 seconds. At five and 10 minutes, suspensions were mixed for 15 s then samples were taken and immediately transferred into phosphate buffer saline solution with 0.28g/ml sodium metabisulfite. Samples were serial diluted and plated on quadruplicated TSA-Rifampicin plates for enumeration.

Results: Both ground and surface water were slightly alkaline (pH 8.13 and 8.01, respectively) and low in turbidity (0.65 and 4.82 NTU, respectively). Both low and high levels of $Ca(ClO)_2$ and PAA solutions inactivated more than 4.5 log CFU/ml of *E. coli* and *Salmonella* cocktails within five minutes at both water temperatures, which exceeded the three-log threshold required by EPA. The total inactivation at 10 minutes was more than six log CFU/ml.

Significance: The results demonstrated adequate sanitizing efficacy of 2 to 4 ppm of $Ca(ClO)_2$ and 6 ppm of PAA in slightly alkaline irrigation water.

P2-137 *Salmonella enterica* Association with Diseased Romaine Lettuce Reduces UV-C Efficacy

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❖ ***Developing Scientist Entrant***

Introduction: Post-harvest sanitation methods such as UV-C irradiation have demonstrated proven effectiveness in reducing pathogen load on leafy greens. However, the internalization of human pathogens into the protective interior of leaves is a concern, and the potential for phytopathogen diseases that afflict leafy greens to promote human pathogen ingression has not been studied.

Purpose: Our study tested the hypothesis that bacterial spot disease of lettuce enhances the access of *S. enterica* to UV-protected areas of leaves and supports *S. enterica* growth.

Methods: A *Xanthomonas vitians* cell suspension (10^8 CFU/ml) or water was syringe-infiltrated into the leaves of four-week-old Romaine lettuce plants. At four days post-infiltration, *S. enterica* Typhimurium was spot-inoculated (10^3 CFU/droplet) onto the surface of pre-treated leaf zones. Viable *S. enterica* populations were measured at 24- and 72-hours post-*S. enterica* inoculation (hpi). Immediately prior to sampling, half of detached leaves were treated with UV-C irradiation (150 J/cm^2) to eradicate surface *S. enterica* cells and measure UV log reduction. Five independent and statistically similar experiments were pooled, using n=30 leaves/treatment group (120 total plants).

Results: At 24 and 72 hpi, *S. enterica* populations on healthy control lettuce plants were significantly reduced ($P<0.003$) to non-detectable levels (LOD = 6 CFU/1 cm leaf disk) by UV irradiation. In contrast, *S. enterica* populations on *X. vitians*-infected lettuce plants were not reduced by UV at 24 and 72 hpi. Furthermore, *S. enterica* populations on *X. vitians*-infected leaves significantly increased ($P<0.0001$) by 3 log between 24-72 hpi, whereas healthy lettuce did not support *S. enterica* population growth. Overall, *X. vitians*-infected lettuce conferred robust protection for *S. enterica* against UV-C irradiation and supported *S. enterica* growth.

Significance: Common phytopathogen diseases such as bacterial spot may reduce the effectiveness of preventative post-harvest sterilization techniques and are a likely risk factor for produce-linked outbreaks in general.

P2-138 Enhancing Microbial Safety of Hydroponic Systems with the Use of Ultraviolet Irradiation

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Introduction: There is much debate around the sources of contamination in hydroponic systems. However, it is widely accepted that once pathogens enter a hydroponic system, the recirculating water can allow them to rapidly spread to an entire crop. Preventative food safety measures for hydroponic systems could potentially reduce the risk of contamination, but more research is needed.

Purpose: The objective of the study was to test the efficacy of an ultraviolet-light (UV) device to reduce the population of Escherichia coli in two types of simulated hydroponic systems.

Methods: Two different types of commercially-available hydroponic systems NFT (Nutrient Flow Technique) and DWC (Deep Water Culture) 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 inoculated with rifampicin resistant Escherichia coli to a concentration of 5-log and treated with a UV-C device (MiniPure MIN-1; 500ml capacity) emitting peak irradiance at 254nm at flow rates of (0,3,6 and 8L/min). Three samples were collected for each treatment and the experiment was repeated twice. The surviving E. coli was enumerated on Tryptic Soy Agar containing rifampicin (80µg/mL). The UV absorbance and transmittance at 254nm of the nutrient solution was measured using a spectrophotometer.

Results: The average UV absorbance (254nm) of the nutrient solution was 0.316. The log reduction with UV treatment of hydroponic nutrient solution at flow rates of 3L/min was significantly ($p<0.05$) higher than the 6 and 8 L/min. At 3L/min, a maximum log reduction of 4.45-log was achieved. However, there was no significant difference in log reduction between 6L/min (3.20-log) and 8L/min (3.11-log) flow rates.

Significance: UV technology can help improve the safety of hydroponic systems and the produce grown using these systems.

Poster

P2-139 Pre- and Post-Harvest Gas Phase Hydroxyl-Radical Treatment to Decontaminate and Extend the Shelf Life of Microgreens in Controlled Environmental Agriculture Operations

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Introduction: Vertical farming is an expending area to serve local markets, counter climate change and promote sustainability. In CEA for microgreens represents a controlled environment although can be susceptible to human pathogens via inputs such as seed and growth substrate. Moreover, a post-harvest wash cannot be applied as this negatively affects the shelf-life which is limited to 5-7 days even without washing. Therefore, there is a need to introduce interventions that can provide preventative controls as pathogen intervention step while extending shelf-life.

Purpose: The following study evaluated gas phase-hydroxyl radical treatment for decontaminating seeds, growing and post-harvest microgreens along with enhancement of plant development and shelf-life of the final product.

Methods: The independent parameters of the gas phase hydroxyl radical treatment were UV-C dose, hydrogen peroxide and ozone concentration. Radish seeds were inoculated with Listeria monocytogenes and treated with hydroxyl-radical treatment. The seeds were germinated on growth substrate and growing plants treated at different stages of development. The plants were harvested at Day 8 and treated with hydroxyl-radical treatment and stored at 4°C. *L. monocytogenes* survivors were determined at each stage of radish plant development and during post-harvest.

Results: Gas-phase hydroxyl radical treatment increased the germination rate of seeds by 15% and reduced *L. monocytogenes* counts by 1.5-2.0 log CFU. *Listeria* became establish on growing radish but could be reduced by 3.45 log CFU by treating growing plants and by 1.85 log CFU at post-harvest. Treating radish microgreens two days before harvest extended shelf-life by 3-5 days. However, treatment plants a day before or following harvest did not extend the shelf-life compared to the non-treated controls.

Significance: Gas phase-hydroxyl radical treatment can be used to successfully inactivate *L. monocytogenes* on seeds and on pre- and post-harvest plants so represents a risk management approach to enhancing food safety of microgreens.

P2-140 Toward Efficient Formulation for Phage-Carrier Biocontrol Agent Against Fire Blight

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Introduction: Fire blight, caused by *Erwinia amylovora*, is a prominent concern for the apple and pear farmers due to its drastic economic effects. Traditionally, streptomycin is used for fire blight management, however, health and environmental concerns urged the need for alternative control methods. Using phage-carrier system of both antagonistic bacterium (*Pantoea agglomerans*) and *Erwinia* phages, as a double sword biopesticide will enhance the biocontrol efficacy.

Purpose: The objective of this project is to develop a protocol for largescale production phage-carrier biocontrol agent and identifying phage receptor(s) on *Erwinia* and *Pantoea*.

Methods: Spray dryer (SD) was used as an economical method for largescale powder production. Different bacterial growth conditions and polymers added to formulate the phage-carrier system were investigated. Phage DNA ejection methods and Proteomics pull-down assay using biotinylated phage were used for phage receptor identification.

Results: An optimized spray drying protocol has been developed and resulted in more than 90% survival rate of phage-carrier system developed using stationary phase growing cells. Although this survival rate is higher than previously published data, further optimization is required to obtain a similar rate with mid-log phase growing cells which will allow for efficient phage infection and propagation on the blossom. The produced powders are stable on shelf-life storage at 4°C for 16 weeks. On the other hand, data of phage receptors identification on both *E. amylovora* and *P. agglomerans* showed that phage is using one of the outer membrane proteins and lipopolysaccharides as receptors. Confirmation of the candidate receptor is ongoing.

Significance: Spray drying of the phage-carrier production is a very promising approach to develop an efficient and feasible biopesticide to mitigate the risk of *Erwinia amylovora*. Furthermore, receptor identification will broaden our understanding of phage-*Erwinia-Pantoea* interaction dynamics.

P2-141 Characterization of *Salmonella* Bacteriophage in Cattle Production Systems in Eastern Island and Two Continental Sites, from Chile

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Introduction: *Salmonella* multidrug resistant (MDR) represents a growing problem for animal, human and environmental health due to its dynamic transmissibility between systems. Therefore, it is essential to identify its presence and dissemination in animal production systems. In nature we sometimes face the difficulty of isolating and characterizing pathogenic bacteria such as *Salmonella*, but fortunately we can isolate and characterize their natural predators, the bacteriophages (phages), and in this case as indicators of their presence

Purpose: In this work we explored the presence of *Salmonella* and phages isolated from fecal and environmental samples from three study areas representing a wide climatic diversity: central Chile (mediterranean), southern Chile (temperate-rainy) and Easter Island (tropical climate).

Methods: A total of 467 samples were analyzed, From each of these samples, isolation by enrichment, host range characterization, comparative genomic and taxonomic analysis were performed.

Results: It was not possible to isolate *Salmonella,* however, it was possible isolate 162 phages (n=18 obtained from S. Infantis host; n=41 from S. Heidelberg host; n=17 from S. Typhimurium host, and n=86 from S. Enteritidis host), with lytic effect on Dublin, Enteritidis and Javiana serovars. Of the total number of phages, nine phages were selected for genetic and taxonomic characterization. Phages selected corresponded to: i) siphoviruses (No:3); ii) myoviruses (No:3); and iii) podoviruses (No:3). This characterization demonstrated a relative diversity of *Salmonella* phages circulating in cattle production systems.

Significance: The study presented included the exploration of three geographically distinct cattle production systems, with differences in management, behavior and type of animals present. It was of great relevance that *Salmonella* was not isolated in any of these systems, but many *Salmonella* phages were isolated, with a lytic effect on *S.* Dublin, *S.* Enteritidis and *S.* Javiana. And in the future they may possibly be used as indicators of the presence of pathogens such as *Salmonella*.

P2-142 Validation of a Bacteriophage Hide Application to Reduce STEC in the Lairage Area of Commercial Beef Cattle Operations

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Introduction: Finalyse is a pre-harvest intervention that utilizes a combination of bacteriophages to reduce incoming *Escherichia coli* O157:H7 prevalence by destroying the bacteria on the hides of harvest-ready cattle entering commercial abattoirs.

Poster

Purpose: The objective of this study was to evaluate the efficacy of Finalyse, as a pre-harvest intervention, on the reduction of pathogens on the cattle hides and the pen floor environments of the lairage area to overall reduce incoming pathogen loads.

Methods: Over 5 sampling events, a total of 300 composite hide samples were taken using pre-moistened swabs, collected before and after the hide wash intervention, at three different timepoints throughout the beginning, middle and end of the production day (n=10 swabs/sampling point/timepoint). A total of 173 boot swab samples were simultaneously taken at the end of the production day, by walking around the pen in a pre-determined "Z" pattern, to monitor the pen floor environment of 3 different areas (outside pens, intervention pen, inside pens) in the lairage area. Specific media combinations, an incubation temperature of 42°C, and incubation timepoints (18-24h) were utilized for each matrix and the prevalence of pathogens was evaluated by using the BAX® System Real-Time PCR.

Results: There was no significant reduction observed for any Shiga toxin-producing *E. coli* (STEC) or *Salmonella* on the hides after the bacteriophage application. *Escherichia coli* O157:H7 and O111 hide prevalence was very low throughout the study, therefore, further analysis was not conducted. However, boot swab monitoring showed a significant reduction for *E. coli* O157:H7, O26, and O45 in the pen floor environment where the Finalyse was applied ($p<0.05$). Prevalence data for each pathogen were evaluated using the Fisher's exact test.

Significance: Using Finalyse in the lairage areas of commercial beef processing facilities can reduce/eliminate *E. coli* O157:H7 in the final meat product, making the beef supply chain safer.

P2-143 Food Safety Culture Excellence through Implementation of GFSI Benchmarked Schemes in Fresh Produce Sector

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Introduction: Access to safe and quality food is of paramount importance and essential requirement for consumers to maintain their health and wellbeing. The meticulous efforts of food producers to demonstrate their commitments to food safety and fulfil customers preferences and expectations can gain more attention if organizations demonstrate well established quality and food safety cultures. Top management commitment and involvement is mandatory to imbed positive food safety culture at all levels in the organizations

Purpose: The purpose of this study was to depict adoption of innovative ideas and reflection of collective attitude, beliefs and behaviours of organizations s top management, managers, supervisors and food handlers towards resolving food safety and hygiene issues and setting contemporary trends which leads towards transforming existing food safety practices into more sophisticated and regimented food safety culture.

Methods: In present study survey of fresh produce facilities and distribution centres on quarterly basis of Dar al Fadhil Group was conducted in kingdom of Saudi Arabia.

Results: Results of this study showed that appropriate trainings, empowering the employees to share their ideas, motivations, strong top management commitments are ways which leads towards transforming existing food safety practices into more sophisticated and regimented food safety culture.

Significance: This study is quite helpful for fresh producers and storage facilities how their objectives turn into reality when they successfully attain certification of their food facilities against GFSI benchmarked schemes by a prestigious GFSI approved certification body.

P2-144 *Staphylococci* in Retail Mushrooms: A Reservoir for the *MecA* Gene

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Introduction: Retail Mushrooms are considered healthy produce and are widely consumed worldwide. Very little research is available that investigates the prevalence, antimicrobial resistance, and virulence of staphylococci in retail mushrooms.

Purpose: To determine the prevalence and genetic diversity of staphylococci in retail mushrooms and to characterize the isolated strains for their virulence and *mecA* gene possession.

Methods: Four hundred and twenty retail mushroom samples were collected from retail stores across Tulsa, Oklahoma area and were screened for the presence of staphylococci. The isolated strains were tested for the presence of *mecA*, and several toxin genes using PCR. One hundred and twenty staphylococcal isolates were subjected to 16S rDNA gene sequencing, molecularly identified, and then subjected to PFGE, MLST, and spa typing. The *mecA* gene was also sequenced when present in these isolates.

Results: The prevalence of staphylococci in retail mushrooms was 71%. Ten different *Staphylococcus* species were detected, and *Staphylococcus fleurettii* was the most abundant species. The prevalence of enterotoxins was relatively low (0.1% to 2.1%), however, the *mecA* gene was detected in 22% of the positive samples. Genetic diversity in the *mecA* gene among staphylococci showed a higher degree of DNA homology among the isolates (97 – 100%), indicating that these isolates can serve as a reservoir of this important gene in the environment. Molecular typing of the identified staphylococci showed high degree of diversity that varied among the mushroom brands which reflects the diverse nature of the composts used to grow these mushrooms.

Significance: The presence of this large number of staphylococci in retail mushrooms is worrisome and could be a food risk for the consumers. The fact that a large portion of the isolated strains carried the *mecA* gene is also very alarming since these staphylococci might serve as a reservoir for the *mecA* gene in the environment.

P2-145 Microbial Population Distinctions between Open Field Grown Versus Controlled Environmental Agriculture Grown Leafy Greens

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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.

Poster

P2-146 Biomapping of Microbial Indicators Using a Mobile Testing Methodology to Assess Agricultural Water System Contamination in a Latin American Farm and Packinghouse

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❖ ***Developing Scientist Entrant***

Introduction: Microbial baselines of indicator organisms can aid in implementation of preventive measures for produce contamination in an agricultural water system (AWS) including mitigation strategies to ensure safety.

Purpose: Develop a biomap of indicator organisms throughout different stages of an AWS used in greenhouse production of bell peppers by the MicroSnap™, a mobile testing methodology.

Methods: Six types of water samples included raw underground, UV treated recirculated, well, well + treated recirculated mixture, and 2 greenhouse irrigation systems. Every repetition, 10 samples/sampling point were taken in 3 replications of the study. One hundred ml water samples were taken with 24oz sterile Whirlpack™ bags. Aerobic (AC), *Enterobacteriaceae* (EB), and *E. coli* (EC) counts were evaluated using the Microsnap™ system following manufacturer's protocols. An ANOVA was used to compare the different stages of the systems for each bacterial indicator using R.

Results: Significant differences were observed throughout the AWS (*P* <0.001). Ultraviolet intervention of recirculated water achieved a significant (*P* <0.001) average reduction of 1.82, 2.4, and 0.7 LogCFU/ml for AC, EB, and EC, respectively. However, when combined with primary source of raw underground water, the native levels of 3.70±0.18 LogCFU/ml (AC) and 3.01±0.16 LogCFU/ml (EB) cross contaminate the treated water and significantly raised (*P* <0.001) EB concentration in the resulting water mixture ready to use for irrigation purposes. Absence of EC was achieved after ultraviolet treatment, but accumulation in the irrigation system was observed by the significant increase in concentration in greenhouse 1 (0.762±0.23 LogCFU/ml).

Significance: Microbiological quality of irrigation systems can be improved if properly measured. Recontamination of treated recycled water is identified as a potential contamination source when mixed with well-underground water. Microbial baselines of water systems can be used for food safety management in produce production systems and the mobile MicroSnap™ methodology showed potential to serve as a testing mechanism in remote locations.

P2-147 Pathogen Prevalence and Correlation to Coliform/*E. coli* Indicators in Maine Wild Blueberry Operations

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❖ ***Developing Scientist Entrant***

Introduction: Wild blueberries are commercially grown only in the far northeast US and Canada. Pathogen prevalence and sources associated with these fruits are not well understood.

Purpose: A comprehensive assessment of indicator populations and pathogens in the preharvest and immediate postharvest environments will facilitate the development of effective education and targeted interventions to improve the safety of wild blueberries.

Methods: During the summer 2022 harvest season, soil and fruit, surfaces of harvesters, bins and conveyor belts were sampled weekly at five working wild blueberry farms. Samples were analyzed by cultural enumeration (for fungi, total aerobes, coliform and generic E. coli) and were subjected to selective enrichment followed by molecular detection of Salmonella, L. monocytogenes and STEC. Quantitative data were analyzed by ANOVA followed by Tukey's HSD, and correlations between these data and pathogen prevalence were assessed by point biserial correlation.

Results: Variability in quantitative data was minimal across site and time, with only yeast levels increasing across the harvest ($p<0.01$). The most commonly identified pathogen in all sample types was STEC, with harvesters testing positive most often, followed by soil, fruit and bins. *L. monocytogenes* was present less often and only in soil and fruit samples. No *Salmonella* was isolated from any sample type. Presence of STEC was more strongly associated with enumerable coliforms ($p<0.001$) than generic *E. coli* (p=0.036). Of samples testing positive for STEC, only 10 (62.5%) and 6 (37.5%) demonstrated the presence of coliform or generic *E. coli,* respectively, in initial analyses.

Significance: Additional data are required to determine the primary pathogen of concern in this crop, but initial findings suggest that STEC is likely to be the focus of future efforts. More importantly, data suggest that routine surveillance for coliform/E. coli may be insufficient to ensure the safety of the crop.

P2-148 A Year-Long Survey of the Microbial Quality of Baby Spinach in the U.S.

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❖ ***Developing Scientist Entrant***

Introduction: In the U.S., spinach is mostly produced in California, Arizona, New Jersey and Texas. Assessing the microbial quality of baby spinach produced in different geographical regions can inform quality assurance practices of industry stakeholders.

Purpose: The objective of this study was to assess the microbial quality of baby spinach over December 2021 to December 2022, from a single processing facility that sources product from two regions (Arizona and California).

Methods: Conventional baby spinach was collected after harvest and packaging, and was shipped overnight to Ithaca, NY. Samples were plated onto petrifilms to determine the aerobic plate count (APC). Harvest samples were tested on arrival. Packaged samples, consisting of washed and unprocessed leaves, were stored at 4˚C on arrival and tested on arrival, day 7 after packaging, and subsequently every 5 or 7 days over 22 or 28 days, respectively. The Baranyi and Buchanan growth models, without a term for lag phase, were fit separately to data from each lot of packaged spinach.

Results: Samples from California had higher microbial loads than those from Arizona. The APC (mean±standard deviation) for harvest samples was 4.8±0.5 $\log_{10}$CFU/g and 4.6±0.9 $\log_{10}$CFU/g, respectively, for spinach from California (n = 12) and Arizona (n = 8). For packaged samples, the APC on day 7 was 7.3±0.6 $\log_{10}$CFU/g and 6.5±0.2 $\log_{10}$CFU/g, respectively, for spinach from California (n = 15) and Arizona (n = 8). The microbial growth rate was 0.21±0.07 $\log_{10}$CFUg^{-1}day^{-1} and 0.23±0.08 $\log_{10}$CFUg^{-1}day^{-1}, respectively, for spinach from California and Arizona. The maximum population level, based on the growth models, was 8.78±0.53 $\log_{10}$CFU/g and 7.68±0.53 $\log_{10}$CFU/g, respectively, for spinach from California and Arizona.

Significance: As location of cultivation may impact the microbial quality of baby spinach, one may want to account for this difference with changes in management practices, such as expediting the product packaging or altering best-by dates.

P2-149 Rain Splash-Mediated Dispersal of *E. coli* from Fecal Deposits in Field-Grown Lettuce

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Introduction: A no-harvest zone around feces in the field could safeguard crops from possible contamination via rain splash, but data on bacterial dispersal are lacking.

Purpose: Assess *E. coli* dissemination from a contamination point source and generate data for determining an adequate no-harvest buffer zone.

Methods: Loose-leaf lettuce 'Magenta' seedlings were transplanted to raised beds with plastic mulch and drip irrigation, in staggered double rows on 30 cm centers. Beds were randomized with four treatments. Eleven and 32 days after transplant (baby and mature lettuce, respectively), 10 g of rabbit manure spiked with 8 logCFU/g *E. coli* TVS353 were deposited in each bed at the 0 m mark, 3 (stale manure) or 0 (fresh manure) days before a rain event. One day after rain, lettuce was collected at 0, 0.3, 0.6, 0.9, 1.2, 1.5 m distances from either side of fecal deposits. Lettuce was stomached in PBS and *E. coli* enumerated with an MPN assay.

Results: *E. coli* TVS353 counts were higher in fresh compared to stale manure plots in both baby ($p<0.001$) and mature ($p<0.01$) lettuce. *E. coli* dispersal from fresh manure was higher in baby than mature lettuce ($p=0.06$). Counts were highest in the baby lettuce, fresh manure trial at 0 and 0.3 m distances (~2.7 logMPN/plant) with a steep decline in subsequent distances of 0.6-1.5 m (0-0.7 logMPN/plant; $p<0.01$). In the mature lettuce, fresh manure trial, *E. coli* counts at the 0 and 0.3 m distances averaged 1.8 logMPN/plant, differing from the 0.6-1.5 m distances (ranging from 0-0.15 logMPN/plant; $p<0.05$). The stale manure treatments yielded low counts and no differences by distance.

Significance: In the field, *E. coli* dispersal by rain was highest when manure was fresh. Dispersal decreased at and beyond the 0.6 m distance from the contamination point source. Data can inform recommendations for no-harvest buffer zones in lettuce fields.

P2-150 Effect of Glandular Trichomes on Epiphytic *Salmonella enterica* Association with Tomato Plants

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Introduction: Trichomes are specialized cells on the surface of leaves, stems and fruit of tomato that are characterized as glandular or non-glandular. *Salmonella* may use non-glandular trichomes as a physicochemical aid for attachment. Glandular trichomes differ by producing volatile organic compounds (VOCs) and other acyl sugar-based secondary metabolites. *Salmonella* interaction with glandular trichomes and its implications for food safety have not been investigated.

Purpose: To investigate the effect that tomato glandular trichomes have on *Salmonella enterica* association with stems and leaves.

Methods: Tomato trichome mutants wo^{mz} (trichome abundant), *af* (anthocyanin free, lacking glandular trichomes), *aa* (anthocyanin absent, carrying glandular trichomes), and their background cultivars 'Ailsa Craig' and 'VF-154', were grown in a greenhouse facility at 27°C) up to 3-weeks post emergence of the first true leaves. Two leaves and a stem section on whole plants were inoculated separately with *Salmonella* serotypes Newport and Javiana at a concentration of ~5.0 log CFU/plant tissue. Plants were incubated at room temperature for 24 hours and bacteria were enumerated by serial dilution and plate counting.

Results: Mutant wo^{mz} yielded lower *Salmonella* counts from leaves inoculated with either serotype ($p<0.05$) and stems inoculated with *S.* Javiana ($p<0.05$), compared to its background cultivar 'VF-145'. Both *S.* Newport and *S.* Javiana leaf counts were 0.35 log CFU/leaf lower than 'VF-145' ($p<0.05$). *S.* Javiana stem counts were 0.84 log CFU/stem lower than 'VF-145'($p<0.05$). Mutant *af* yielded 0.35 log CFU/leaf higher ($p\leq0.05$) *S.* Javiana counts on leaves compared to its background 'Ailsa Craig' and 0.21 and 0.22 log CFU/stem ($p\leq0.05$) higher *S.* Newport counts on stems compared to *aa* and 'Ailsa Craig', respectively ($p\leq0.1$). No significant difference was detected in *S.* Newport association on leaves between *af, aa* and 'Ailsa Craig'.

Significance: Data suggest that glandular trichomes may play a role in modulating the association of *Salmonella* with tomato stems and leaves.

P2-151 The Role of Alterations in the Leafy Green Phyllotelma on Foodborne Virus Adhesion and Inactivation on Romaine Lettuce and Spinach Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: The plant surface waterscape, termed the phyllotelma, is influenced by plant surface topography, yet the impact of the phyllotelma on foodborne pathogen interactions with produce remains unclear.

Purpose: Determine the role of the phyllotelma on foodborne virus adhesion to leafy green surfaces and its impact on viral inactivation by sanitizers.

Methods: Polydimethylsiloxane (PDMS) replicasts (topomimetic artificial leaf surfaces) of 15- and 45-day old leaves of spinach and romaine lettuce plants were generated with flat PDMS replicasts (no surface topography) included as controls. Half-inch diameter coupons of adaxial and abaxial surfaces of replicasts and fresh leaves of the same age were inoculated with 10^5 PFU of Tulane virus (TV) in triplicate. Inoculated coupons were submerged in a sanitizer solution (50 ppm sodium hypochlorite (SH), 200 ppm SH, 20 ppm peracetic acid (PAA), or 80 ppm PAA) or control (ultrapure water) and agitated for 30 seconds before adding a 10% sodium thiosulfate solution as a sanitizer quencher. Coupons were then transferred to a rinse solution containing 0.05% Tween20 in phosphate buffered saline. TV titer was determined by plaque assay in both the sanitizer and rinse solutions.

Results: Results using PDMS replicasts of romaine lettuce and spinach leaves indicated no significant difference in virus recovery or inactivation based on leaf age or axis. Treatment with 50 ppm SH resulted in a 2.5-3 log reduction in TV and >5-log reduction was observed after treatment with 200 ppm SH. The highest concentration of PAA (80 ppm) resulted in a 1.5 log reduction in TV. No significant differences in results for PDMS replicasts and fresh leaves were observed.

Significance: Alterations in the phyllotelma induced by variations in surface topography (leaf age, leaf axis) did not influence viral inactivation by sanitizers. Moreover, the PDMS replicasts produced similar results to fresh leaves, indicating the utility of this model system.

P2-152 Survey of Small Local Produce Growers' Knowledge of Microbial Contamination and Perception of the Triple-Wash Method at Farmers' Markets

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Introduction: Farmers markets (FM) meet consumers' desires for locally-grown fresh produce. It is imperative that farmers understand the microbial risk associated with bacterial contamination on produce. The triple-wash method (TWM) is effective at reducing microbial contamination on produce.

Purpose: To determine small local produce growers' awareness and attitude toward microbial contamination risks and TWM.

Methods: Forty Surveys were conducted (face-to-face questionnaire interview) at five FMs in WV, one FM in Ohio, and two FM in PA from May thru November in 2022. Questions included basic information of age, gender, and education level, awareness of microbial contamination risks, source of irrigation water, washing methods, knowledge, and interest in TWM implementation and training. Data were analyzed using JMP software to compare the difference between different locations. Chi-square tests of independence were employed to examine bivariate relationships between categorical variables ($P=0.05$).

Results: Survey response rate is 93% (65.6% males, 28.1% females, 6.3% other). 21.9% of participants implement no washing strategies for their produce and 65.6% say they have never heard of TWM. 75% of farmers reported that they would use TWM if it were affordable and reduced bacteria. The most important factors when deciding to utilize TWM was easy implementation (53.1%) and low cost (40.6%) ($P<0.05$). If TWM reduces microbial risks by 5%, most farmers (37.5%) were unsure of what amount they would accept for an increase in production cost without changing prices of their products. Similarly, 46.8% of farmers were not sure how much they would increase the price of their products if TWM increased production costs by 5%. 31.3% of participants are interested in attending TWM training from GAP/FSMA ($P<0.05$).

Significance: Understanding FM vendors' perceptions and current wash methods allow local state government agencies to make better informed decisions regarding food safety policies related to produce safety.

P2-153 Preparation Methods and Perceived Risk of Foodborne Illness Among Consumers of Prepackaged Frozen Vegetables – United States, September 2022

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Introduction: *Listeria monocytogenes* causes listeriosis, a serious infection. For persons at higher risk for listeriosis, infections commonly result in hospitalization and sometimes death. The first reported *Listeria* outbreak linked to frozen vegetables occurred in 2016 and resulted in three deaths. Many frozen vegetables are intended to be consumed after cooking. However, data on consumer behavior are sparse.

Purpose: We characterized consumers' demographic characteristics, perceptions of listeriosis risk from frozen vegetables, and preparation methods of frozen vegetables including among people with cancer.

Methods: From September 1, 2022 thru September 24, 2022, Porter Novelli Public Services conducted the FallStyles survey using the Ipsos Knowledge-Panel. People with cancer were those who self-reported either skin cancer or other cancer during the past year. Data were weighted to be representative of the U.S. population. Point estimates and 95% CIs were calculated and differences between respondents were determined using Wald chi square tests. P-values <0.05 were considered statistically significant.

Results: Among 3,008 respondents reporting a preparation and consumption method for frozen vegetables, 8.7% (95% CI = 7.4% to 10.0%) reported ever consuming the product raw. For respondents who consumed them raw, the most commonly reported preparation method was adding them directly to a blender for smoothie or juice (4.9%; 95% CI=4.0 to 5.8%). 34.1% (95% CI=32.2% to 35.9%) of respondents agreed that prepackaged frozen vegetables can be contaminated with germs (like *Salmonella*, *E. coli*, and *Listeria*), with a greater proportion of people with cancer disagreeing compared to those without cancer (32.5% vs 23.4%, *P*=0.041).

Significance: Almost 10% of consumers reported consuming raw frozen vegetables, most commonly in juices or smoothies. Only one-third of consumers thought frozen vegetables could contain *Salmonella*, *E. coli*, and *Listeria*. Understanding consumers' consumption, preparation, and risk perception of frozen vegetables can inform prevention strategies. Reducing contamination of frozen vegetables before they are sold to consumers is important.

P2-154 Evaluation of Knowledge Gained from Food Safety and Good Agricultural Practices Educational Material for Kentucky Growers

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Introduction: The fresh produce rules under the Food Safety Modernization Act (FSMA) establish mandatory food safety guidelines for produce growers. There is a need for crafting educational and outreach materials to train fruit and vegetable growers on food safety and Good Agricultural Practices (GAPs).

Purpose: The objective of this research was to use research-based information to develop fresh produce factsheets and to evaluate changes in farmers' knowledge on the safety and GAPs of fresh produce associated with foodborne outbreaks.

Methods: Six fact sheets for tomatoes, lettuce, spinach, alfalfa sprouts, squash, cucumbers, and melons were developed. The fact sheets had a standardized design with 6 major sections in each fact sheet and covered general commodity information, pathogenic behavior in commodity, harvest considerations, foodborne illness outbreaks associated with commodity, and storage and cooling conditions. To evaluate the fact sheets, a questionnaire was developed and administered at farmers' markets in Kentucky to small-scale farmers (n=49) who grew and sold fresh produce. The questionnaire utilized a 5-point Likert scale with 1=no knowledge, 2=slightly knowledgeable, 3=neutral, 4=moderately knowledgeable, and 5=extremely knowledgeable.

Results: Pre- and post- knowledge of the completed fact sheets was assessed. The results showed a significant (*P*< 0.05) increase in produce growers' knowledge on GAP-related activities. The fact sheets significantly (*P*< 0.05) increased knowledge about foodborne illness outbreaks, pathogenic behavior, and GAPs and the key elements of food safety practices.

Significance: The study has demonstrated that commodity-specific fact sheets were a highly effective and efficient way to educate small-scale Kentucky farmers on fresh produce safety and GAPs. This study provided evidence that free and easily distributed educational handouts can improve knowledge among farmers who are receptive to education on GAPs.

P2-155 Evaluating the Consumers' Acceptability of QR-Labeled Apple Fruit

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce is traditionally labeled with plastic price lookup (PLU) stickers. However, PLU stickers are environmental hazards (glue and plastic), and frequent detachment of those stickers disrupts their traceability. Engraving Quick Response (QR) codes on produce surface is a novel method of produce labeling. Research has been conducted on determining its impact on microbial attachment and postharvest quality. However, research information on consumers' perception and acceptability are unknown.

Purpose: To determine the consumers' perception and acceptability of the QR-labeled 'Red Delicious' apple (*Malus pumila*)

Methods: The 'Red delicious' apples were procured from a local grocery store and were printed with a QR code (Trotec Speedy 300 CO_2 laser engraver) followed by the application of edible wax. This study was conducted as an onsite survey (n=75) between the age group 18-64. The study had three treatments: 1) Apple with QR-code, 2) Apple with PLU plastic sticker, and 3) non-treated control. A set of three treatment samples were given to the respondents, and observation questionnaires were asked to fill up. Data was tested for significance at p<0.05.

Results: Based on overall physical appearance revealed that before providing information on the technology, consumers ranked QR labeled apples as the lowest group (p<0.05), followed by sticker-labeled and no label. In contrast, after reading some information, the ranking scores from the same respondents were statistically similar (p>0.05). About 56% of the respondents did not have any concerns about QR-labeled apples and 52% agreed that all possible produce should be QR-labeled. Similarly, respondents' preferences for QR-labeled and PLU-labeled were 52% and 48%, respectively. Providing information on the technology improved the consumers' perception and overall liking and thus warrants need for education programs.

Significance: QR labeling has the potential to be an alternative to the PLU sticker to improve traceability in apples/fresh produce.

P2-156 Produce Safety: Enhancing Risk Assessment at the Field Level – Application of Tools during Pre-Harvest, Harvest, and Post-Harvest to Mitigate Food Borne Pathogens in Supply Chain

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Introduction: Produce is a high-risk food item with no kill step from seed to table, therefore the need for robust, rigorous, easily implementable, and effective risk assessment tools at all levels of harvesting to ensure a safe food supply through enhanced tools for mitigating the potential for food borne micro-organisms.

Purpose: To educate, share best practices, and create a culture in the industry of collaboration, inclusion, and elevating our ability to ensure a safe food supply to our consumers.

Methods: Risk assessments in the agricultural industry are ultimately people-based, therefore there is a need for effective, but easily implementable and communicated techniques for risk assessment across a variety of harvest areas including harvest crews, pre-harvest, harvest, and post-harvest con-

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ditions. We have developed a hybrid of tools established by credible organizations and companies such as McDonald's (Global GAP+ appendum), Primus, LGMA, FSMA FSVP, as some examples of tools used to create a model for our organization. The tools are easily related to by field staff and crews, while focusing on behaviors and system processes.

Results: As a global organization (US$4B) that leverages both open and close fields (greenhouses), we have results from audits where we regularly score in the 90s for these field audits, with no major product withdrawal event (Fresh Del Monte product) in the past three years. While we are a strong industry player, we also want to enhance an industry that still strives for better food safety.

Significance: In the United States there are more than 200 outbreaks/recalls related to a variety of produce items. With no kill step involved in our process to ensure our fresh business and the health and wellness category, ensuring rigorous and disciplined approach to risk assessment at the field level is business and industry critical. Our call to action is more enhanced risk assessment tools.

P2-157 Efficacy of Commercially Available Sanitizers to Prevent Cross-Contamination during Simulated Postharvest Washing of Cucumbers

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❖ ***Developing Scientist Entrant***

Introduction: Recirculated or batch water used for postharvest washing of produce accumulates organic matter, reducing the efficacy of sanitizers used to prevent cross-contamination. Hence, it is important to understand sanitizer efficacy at different chemical oxygen demand (COD) levels commonly encountered in commercial operations.

Purpose: To evaluate the combined effect of COD and sanitizer concentration for reduction of *Escherichia coli* TVS353 as a surrogate for bacterial foodborne pathogens on inoculated cucumbers and prevention of cross-contamination to uninoculated cucumbers and water during simulated immersion washing.

Methods: Two COD levels (300 and 2500 ppm) and four sanitizer concentrations (0, 20, 40, and 80 ppm) of free chlorine or peroxyacetic acid were evaluated. Containers were filled with 10L of sterile deionized water and adjusted to the appropriate treatment conditions. Slicing cucumbers were spot inoculated with *E. coli* TVS353 (~9 to 10 log CFU/cucumber) and allowed to air dry. For each treatment combination, one inoculated and nine uninoculated cucumbers were immersed in the water with a contact time of 1 min prior to quenching of sanitizer activity and enumeration of viable populations. Percent transfer to produce and water as well as log reduction on inoculated produce was calculated. Six biological replicates were performed for each treatment combination.

Results: The 2500 ppm COD level with 20 ppm free chlorine treatment combination had significantly higher transfer of *E. coli* to uninoculated produce (1.1×10^{-6} %) compared to other treatment combinations with sanitizer present ($P<0.05$); no significant differences were seen for transfer to water for this treatment ($P>0.05$). All other sanitizer treatments were not significantly different ($P>0.05$) when evaluating the percent transfer to water or produce and log reduction on inoculated produce.

Significance: This study will aid industry in establishing science-based best practices for recirculated or batch postharvest washing water under varying concentrations of COD for reducing bacterial transfer.

P2-158 Effect of Drop Height on Internalization of Generic *Escherichia coli* in Fresh Cucumbers

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❖ ***Developing Scientist Entrant***

Introduction: The FDA Food Safety Modernization Act Produce Safety Rule prohibits harvesting dropped covered fresh produce due to potential microbial contamination.

Purpose: This study aimed to determine the effect of drop height on internalization of generic *Escherichia coli* in fresh cucumbers.

Methods: Fresh mini cucumbers were obtained from a commercial greenhouse. Five drop heights (0, 1, 2, 4, and 6 ft) were assigned to four cucumbers each (n=20) and dropped through height-controlled PVC pipes. Following a completely randomized block design, cucumber samples were dip inoculated with a 7.0±0.2 log CFU/100 mL rifampicin-resistant (80 ppm) generic *Escherichia coli* (gEC; TVS 353) solution (5 min, 150 RPM). Generic *E. coli* was enumerated on the surface of each cucumber sample using IDEXX Quanti-Trays. Cucumber samples were then surface sterilized (70% ethanol, 5 min, 150 RPM), peeled, stomached, and enumerated to determine internalized gEC. Cucumber samples were enriched using methods outlined in the FDA BAM. Significant differences ($P \leq 0.05$) were evaluated by Tukey's HSD test in JMP Pro 16.

Results: Excessively damaged cucumbers that did not represent marketable produce (i.e., broke into > 2 separate pieces) were excluded from analysis. Cucumber dropped from 6ft (100%) and 4 ft (25%) were unmarketable. No significant differences were observed between drop heights (0, 1, 2, 4 ft) and gEC counts on the surface of cucumber samples ($P>0.05$, range: 4.1-4.5 log MPN/cucumber). All cucumber samples, post-surface sterilization, were below the limit of detection (<0.0 log MPN/cucumber) for gEC at all four remaining drop heights; however, 100% of enrichments were positive.

Significance: Results confirm dropped produce should not be harvested. Data showed drop height did not affect gEC counts on the surface of cucumbers or population internalized. Given contamination post-surface sterilization, harvested dropped cucumbers could pose a downstream food safety risk.

P2-159 Survival of *Salmonella enterica* and *Enterococcus faecium* on Onion Handling Surfaces

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Introduction: Currently there is limited knowledge on the survival of bacteria on surfaces during postharvest handling of dry products such as onions. Extended survival of microorganisms, coupled with a lack of established regular validated cleaning or sanitation methods in the dry fresh produce industry, could enable cross-contamination of these products.

Purpose: The aim of the study was to evaluate the survival of *Salmonella enterica* and potential surrogate *Enterococcus faecium* on typical onion handling surfaces with different organic loads and under dry conditions.

Methods: Rifampin-resistant *Enterococcus faecium* NRRL B-2354 and a five-strain cocktail of *Salmonella* suspended in 0.1% peptone or onion extract were inoculated onto polyurethane (PU) and stainless steel (SS) coupons (2 × 2 cm), at high, moderate, or low (7, 5 and 3 log CFU/cm^2) levels. The inoculated surfaces were stored at ~34% and 21°C for up to 12 weeks. Triplicate samples were enumerated at regular intervals in replicate trials. Samples were enriched when populations fell below the limit of detection by plating (0.5 log CFU/cm^2). Scanning electron microscope (SEM) was used to observe the cell morphology on the coupons.

Results: Reductions of less than 2 log CFU/cm^2 of *E. faecium* were observed on PU and SS over 12 weeks at all inoculum levels and with both inoculum carriers. In 0.1% peptone, *Salmonella* populations declined by 2 to 3 log CFU/cm^2 over 12 weeks; at the low inoculum level, *Salmonella* could not be recovered by enrichment at 12 weeks. Survival of *Salmonella* was significantly ($P < 0.05$) enhanced over 12 weeks of storage when suspended in onion extract, where cells were covered by a layer of onion extract.

Significance: Onion juice improved the survival of *Salmonella* under dry conditions; *E. faecium* might have utility as a conservative surrogate for *Salmonella* when evaluating microbial survival on dry food-contact surfaces.

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P2-160 Transfer of *Enterococcus faecium* and *Salmonella enterica* in Yellow Onions during Simulated Postharvest Handling

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Introduction: Bacterial transfer during postharvest handling of fresh produce provides a mechanism for spreading pathogens, but there is a lack of knowledge about the risk factors for bacterial transfer in dry packinghouse environments.

Purpose: The aim of the study was to investigate factors that influence the transfer of bacteria between yellow onions and dry food-contact surfaces.

Methods: Rifampin-resistant *Enterococcus faecium* NRRL B-2354 or a five-strain cocktail of *Salmonella* were inoculated onto onions or polyurethane (PU) surfaces (2 × 2 cm) at 7 log CFU/cm^2 (high) or 5 log CFU/cm^2 (moderate) using 0.1% peptone, onion extract, or a soil and water mixture as inoculum carriers. After the inoculum had dried, transfer from inoculated to uninoculated surfaces was conducted using a texture analyzer to control the contact force and time. After a preliminary assessment, a combination of five repeated 10 N, 30 s contacts was selected to understand other factors, including transfer direction(onion-to-PU vs PU-to-onion), recipient surface (PU and stainless steel [SS]), inoculum levels, bacterial species, and inoculum carriers (n = 9–18). Percent transfer rate (TR) was calculated as a ratio of the population on the recipient surface to the source population.

Results: The TR of *E. faecium* was impacted by transfer direction (4% vs 0.4%), but was not influenced by recipient surface or inoculum level. The TR of *E. faecium* (4-5%) was significantly high than *Salmonella* (0.5 to 0.6%) at high inoculum level; differences in TR for *E. faecium* (3-5%) and *Salmonella* (6-11%) were not significant at moderate inoculum level. The transfer was significantly impacted by inoculum carrier, with transfer rates of 61% (onion), 1.6% (peptone), and 0.30% (soil).

Significance: Bacterial transfer during onion handling is significantly dependent on transfer direction, inoculum level, bacterial species, and inoculum carrier.

P2-161 Transfer of *Escherichia coli* O157:H7 to Romaine Lettuce Heads during Simulated Field Harvest

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Introduction: Romaine lettuce has been implicated in numerous recurring foodborne outbreaks involving Shiga toxin-producing *Escherichia coli* (STEC). Knives, used during field harvesting, may serve as a vehicle for contamination.

Purpose: To evaluate the transfer potential of *E. coli* O157:H7 from contaminated commercial lettuce knives to romaine heads during harvesting.

Methods: Romaine lettuce plants (cultivar 'Green Towers') were grown from commercial seed and maintained in a BSL-3P phytotron greenhouse. Sterilized blades of field lettuce and harvesting knives (Arizona Bag Company, LLC; catalog #4125) were artificially contaminated with 1 ml of an O157:H7 culture at two inoculum concentrations (ca. 5.4 or 2.4 log CFU/ml) and allowed to dry for 2 hours in a biosafety cabinet. Successive harvesting of up to 14 mature romaine lettuce plants (52 days post planting) was performed with O157:H7-contaminated knives. Each experiment was conducted in triplicate. Romaine heads [n=91; average weight (302.8g)] were analyzed for presence or absence of *E. coli* O157:H7 by enrichment using a modified FDA-BAM method, and the Pearson Chi-Square test was used to determine significant differences in contamination by inoculum concentration.

Results: Field knives inoculated with O157:H7 at 5.4 or 2.4 log CFU/ml, then repeatedly used to harvest lettuce heads, transferred pathogen to 10 and 3 consecutive harvested heads, respectively. Non-sequential contamination was observed on the last harvested head for both inoculum levels. Of the total heads harvested (5.4 log CFU/ml or 2.4 log CFU/ml), 72.5% (29/40) and 17.5% (7/40) were positive for O157:H7, respectively. The number of O157:H7-positive heads was significantly greater for the higher inoculum concentration (X^2=24.444, P<0.0001).

Significance: These results demonstrate that commercial field knives contaminated with O157:H7 may contribute to cross-contamination during harvesting and supports the importance of developing adequate cleaning and sanitation protocols for harvesting tools.

P2-162 Survival of Generic *E. coli* in Soil Amended with Biological Soil Amendments of Animal Origin (BSAAO)

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❖ ***Developing Scientist Entrant***

Introduction: The application of BSAAOs, such as composted poultry litter (CPL) or heat-treated poultry pellets (HTPP), can improve soil health; however, their potential impact on the survival of bacterial pathogens is of particular concern for fresh produce safety.

Purpose: This study aims to observe the effect of BSAAOs on the survival of *E. coli* in Florida soil.

Methods: Twelve raised bed plots (3 m^2) were amended with 680g/plot (approx. 1 ton/acre) of composted poultry litter (CPL) or heat-treated poultry pellets (HTPP). Negative (no *E. coli*/no BSAAO) and positive (*E. coli* /no BSAAO) plots were also prepared and evaluated (n=3 per treatment). The study was conducted on a research farm in Live Oak, Florida. Plots were spray-inoculated with 1 L of rifampicin-resistant *E. coli* TVS353 inoculum (10^8 CFU/ml) and hand-tilled. Soil samples were collected at specified time intervals (0, 1, 3, 7, 14, 28, 56, 84, 112, 140 days) and enumerated on tryptic soy agar supplemented with 80 ppm rifampicin (TSAR). When plate counts fell below the limit of detection (LOD, 0.70 log CFU/g), an MPN procedure was utilized.

Results: E. coli declined rapidly from 5.22±0.27 log CFU/g to below LOD using the plate count method in unamended, positive control plots after 14 days. For CPL-amended plots, *E. coli* was detected until day 56. *E. coli* survived significantly (P<0.05) longer in HTPP-amended plots compared to both the CPL and the unamended, positive control plots. *E. coli* was still recoverable in HTPP-amended plots on the 140-day sampling period (1.5 log CFU/g). No *E. coli* was detected (<0.7 log CFU/g) in the negative control plots.

Significance: Soils amended with BSAAOs extended the persistence of *E. coli*, with survival >140 days in HTTP-amended plots. These findings facilitate the development of guidelines regarding the interval between BSAAO application and the harvest of fresh produce.

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P2-163 The Transfer of Generic *E. coli* to Onions during Field Trials and Determining Its Survival in Post-Harvest Storage Studies

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❖ ***Developing Scientist Entrant***

Introduction: Onions have been implicated in foodborne outbreaks. Understanding pre-harvest contamination routes and pathogen fate during storage before consumption is important to mitigate risk. Transfer of pathogens from soil to onions is not well characterized.

Purpose: The aim of this study was to assess *Escherichia coli* transfer from inoculated soil to cultivated onions in an on-farm study. Survival of *E. coli* on onions during post-harvest storage was also examined.

Methods: Raised bed plots (3 m²) were prepared (n=3) and inoculated with 1 L of *E. coli* TVS353 inoculum (10^8 CFU/ml) and hand-tilled. Sweet onions were transplanted 14 days post-inoculation and removed from the ground after 133 days. Onions were then allowed to cure in the field, on the soil, for 14 days. Five composite samples (two onions/sample) per plot were tested for *E. coli* before and after curing using a MPN method. For the post-harvest storage study, onions were spot inoculated with 100 µl of *E. coli* TVS353 (10^9 CFU/ml), air-dried (60 min) and stored at 20ºC or 30ºC. Samples were enumerated using an MPN procedure after 0, 8, 24, 36, 72, and 96 h.

Results: For field onions, before curing, 15/15 samples tested positive for *E. coli* TVS353 (1.89 log MPN/onion). After curing, only 1/15 samples tested positive (0.47 log MPN/onion). During the storage study, *E. coli* populations on onions significantly (P<0.05) declined over time at 20 and 30°C. At 30°C, a 3-log MPN reduction was achieved after 24 h, while at 20°C it took 72 h to achieve the same reduction. After 96 h, a total reduction of 3.5-log MPN/onion was achieved at 30°C, compared to only 3 log at 20°C.

Significance: Extended survival of *E. coli* in soils promoted transfer to sweet onions, but prolonged survival on onions was not observed in the field or during post-harvest storage.

P2-164 Survival of Generic *Escherichia coli* on Different Harvest Bag Material-Types

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❖ ***Developing Scientist Entrant***

Introduction: Harvest containers are a source of microbial contamination when they are not cleaned; thus, data on the microbial quality of these surfaces can inform sanitation best practices.

Purpose: The objective was to evaluate generic *Escherichia coli* survival on three different harvest bag material-types (100% each canvas, nylon, and cordura).

Methods: Coupons of each material-type were inoculated with a single strain of rifampicin-resistant (80ppm; R) generic *E. coli* (TVS353) at ~7.3±0.1 log CFU/coupon. Coupons were air-dried for 90min, and held at ~22°C with 30 or 80% relative humidity (RH). *E. coli* were enumerated at 11 time-points: 1.5, 4, and 8 h, and 1, 2, 3, 7, 14, 30, 60, and 90 days post-inoculation (dpi). Coupons were subjected to rub-shake-rub for 60s with 0.1% peptone, and plated in duplicate on selective (MacConkey-R) and non-selective (Tryptic Soy Agar-R) media (n=9). Significant differences (P≤0.05) were evaluated by Tukey's HSD test in R-Studio (version 4.1.1).

Results: At 30% RH, significant *E. coli* reductions of 1.2±0.4 and 1.2±0.3 log CFU/coupon were observed at 2 dpi on nylon and cordura, respectively (P≤0.01). On canvas, significant reductions were not observed until 3 dpi (0.8±0.3 log CFU/coupon; P=0.01). By 90 dpi, *E. coli* were undetectable on nylon, but detectable on canvas and cordura. At 80% RH, significant *E. coli* reductions of 0.9±0.2, 1.0±0.4, and 1.3±0.6 log CFU/coupon were observed at 2 dpi on nylon, canvas, and cordura, respectively (P≤0.01). By 90 dpi, *E. coli* were undetectable across all material-types. The relationship between dpi and *E. coli* was fitted using a segmented linear model (R^2=0.92), with breakpoint at 0.5d (CI: 0.35, 0.58) and 20.1d (CI: 18.3, 21.8).

Significance: Findings show survival of *E. coli* is impacted by RH, time-point, and material-type, with canvas exhibiting the slowest die-off. Frequent cleaning is recommended as *E. coli* survived on across all harvest bags material-types at >30d.

P2-165 Transfer of Generic *Escherichia coli* from Different Harvest Bag Materials to Apples

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❖ ***Developing Scientist Entrant***

Introduction: Cross-contamination of food contact surfaces to fresh produce can result in foodborne outbreaks and recalls.

Purpose: This research quantifies the transfer rate of generic *Escherichia* (gEC) from different harvest bag materials to unwaxed apples.

Methods: Four different materials (canvas, cordura, leather, and nylon) were cut to 5x5 cm coupons, and inoculated with a single strain of rifampicin-resistant gEC (TVS353). Materials were air-dried for 1 or 4 h. Concentrations of gEC on materials post-drying was ~7 log CFU/coupon. 'Red delicious' unwaxed apples from a commercial packinghouse were weighed and placed on inoculated materials for 5- or 25-min contact times with 0 or 5 lbs (0.1kg/cm²) weights. Coupons and apples were sampled using rub-shake-rub for 60s with 0.1% peptone+0.1% Tween 80 and plated in duplicate on selective (MAC-R) and non-selective (TSA-R) media (n=9). Transfer rates were expressed as log % transfer, and a regression model was fitted (P≤0.05).

Results: Contact time (P=0.54) and pressure (P=0.33) did not significantly impact transfer. Inoculum, dry-time and applied weight significantly influenced the log % transfer of gEC (P≤0.001). Log percent transfer of gEC was significantly less to leather and nylon vs. canvas (P<0.001). Interaction effects of dry-time x weight applied, and inoculum dry-time x material was significant (P<0.05). Samples dried for only 1h transferred more gEC to apples (-1.67 to1.57 log % transfer), vs. when a 4h dry-time was used (-3.39 to -1.06 log % transfer). The log % transfer at 1h dry-time varied by material-type, pressure, and contact-time.

Significance: Longer contact time does not increase transfer. Placing apples in contaminated bags may result in cross-contamination. These findings will inform risk management decisions for apple growers.

P2-166 *Salmonella* Cross-Contamination Risks between Tomatoes and Harvest Bins during Harvesting

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❖ ***Developing Scientist Entrant***

Introduction: Tomatoes have been frequently associated with salmonellosis in the U.S.

Purpose: The purpose of this study was to evaluate *Salmonella's* transfer potential between three common harvesting bin materials and tomatoes.

Methods: Tomatoes or HDPE, wood, or cardboard coupon (5x5 cm) were spot inoculated with a *Salmonella* cocktail (10^5) and dried for 1h. Uninoculated tomatoes or cartons were placed into contact with inoculated items; a 1lb weight was placed on top of each tomato to mimic the pressure of a 25 lb bin,

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and left for 10min, 3, 6, and 24h. At each sample time, bins and tomatoes were placed into separate WhirlPak bags with 0.1% peptone. A shake, rub, shake, (30s each) was followed by dilutions, plating onto non-selective media with Rifampicin, and incubation (35°C, 24h). The experiment was replicated 3 times with 3 samples (n=9). Transfer coefficients (TC) were calculated by dividing the CFU/mL *Salmonella* on the uninoculated surface by the CFU/mL *Salmonella* on the inoculated surface and reported as log %TCs.

Results: *Salmonella* transfer between tomatoes and HDPE were significantly higher ($P \leq 0.05$) than between tomatoes and wood or cardboard; tomato to HDPE: 1.4 to 4.2, and HDPE to tomato: 0.2 to 2. Transfer between tomatoes and cardboard ranged from -1.3 to 2 (tomato to cardboard) and -0.34 to 1.5 (cardboard to tomato); no transfer was seen after 6h in either case. Transfer from tomatoes to wood ranged from-1.9 to 1.5; transfer did not increase after 3h. Transfers from wood to tomato ranged from -0.6 to 1.3; transfer did not increase after 10min.

Significance: TC between tomatoes and HDPE is higher than transfers from cardboard and wood. HDPE is considered a more cleanable and sanitizable surface than cardboard or wood but may result in higher cross-contamination if not properly managed.

P2-167 Bacterial Transfer during Blueberry Harvest

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❖ ***Developing Scientist Entrant***

Introduction: Inspections of blueberry farms have demonstrated regulatory emphasis on frequency and adequacy of cleaning and sanitation of harvest containers. Data are needed to understand transfer of microorganisms to and from produce and food contact surfaces to support risk analysis efforts and demonstrate efficacy of cleaning and sanitation practices for the produce industry.

Purpose: Quantify bacterial transfer between blueberries and harvest buckets to evaluate risk of cross-contamination during harvest activities.

Methods: Fresh blueberries (1 kg) were inoculated with rifampicin-resistant *Escherichia coli* (~6.0 log CFU/g), added to a plastic harvest bucket (4778 cm^2 interior), agitated (5 min) and removed. A second batch (1 kg) of uninoculated blueberries was then added to the bucket, agitated (5 min) and removed. Inoculated and uninoculated blueberries and swabs (3M sponge) of the bucket interior were enumerated for *E. coli* on eosin methylene blue agar + 50 ppm rifampicin, incubated at 37°C, for 24 hours. Experiments were replicated three times.

Results: Addition and agitation of inoculated blueberries (6.1±0.1 log CFU/g) to the harvest bucket led to surface contamination of 4.2±0.2 log CFU/cm^2. Subsequent transfer of *E. coli* from the contaminated bucket to uninoculated berries averaged 3.8±0.1 log CFU/g. Transfer rates from contaminated berries to clean buckets and from contaminated buckets to uninoculated berries were 6.2% and 9.1%, respectively. Collective microbial transfer rate from heavily contaminated blueberries to clean berries via contaminated buckets is estimated at 0.6%. Transfer rates were consistent across three independent experiments.

Significance: These data can be used to model and predict cross-contamination risk as a function of frequency of cleaning and sanitation of harvest equipment. Recognizing that harvesters are instructed to never harvest conspicuously contaminated berries, pathogen load entering the bucket is likely to be much lower than contamination levels used in this study.

P2-168 The Effect of Organic Matter on Antimicrobial Activity of Chlorine in Post-Harvest Wash Water to Control *Listeria monocytogenes*

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Introduction: The use of chlorine in wash water is a common intervention method for pathogen control in post-harvest processing of fresh produce, but its efficacy is affected by organic matter.

Purpose: This study investigates the effect of fresh produce-associated organic matter in quenching antimicrobial activity of chlorine.

Methods: Produce wash water of varying levels of chlorine (20, 40, and 80 ppm) were prepared using 3 concentrations of organic matter (OM) in the form of soil (SOM) and fruit (prepared as peach puree and denoted as FOM): 0.1%, 1%, and 10% (w/v). Each water sample (100 ml) was inoculated with 1 ml of a 5-strain cocktail of rifampicin-resistant *L. monocytogenes* (9.38±0.12 log CFU/ml) and exposed to the treatment for 0, 5, 10, and 20 minutes. Aliquots for each time treatment were neutralized with D/E broth (1:1 v/v). Quantification followed using 3 biological and technical replicates each. Significant differences were analyzed using ANOVA.

Results: The highest concentration of 10% (w/v) SOM and FOM were found to significantly inhibit chlorine activity wherein *L. monocytogenes* was still detected ($p<0.05$). The wash water with 40 ppm chlorine and 10% FOM showed significantly higher recovery of *L. monocytogenes* at an average of 6.59±0.45 log CFU/ml compared to all the other samples treated ($p<0.05$). For SOM, treatment with 40 and 80 ppm chlorine showed significantly higher reductions in population where *L. monocytogenes* was not detected ($p<0.05$). For FOM (10%), treatment with 80 ppm chlorine significantly reduced the bacterial population where 0.77±0.63 log CFU/ml was recovered ($p<0.05$). The time of chlorine exposure was found to not significantly vary with either SOM and FOM in wash water ($p<0.05$).

Significance: The findings for this study can help suggest the optimal concentrations of chlorine that would inhibit bacterial survival in the presence of organic matter.

P2-169 Anti-*Listeria* Efficacy of a Peroxyacetic Acid-H_2O_2 Mixer in Bacterial Buffered Solution and on Peppers

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Introduction: West-Virginia-Small-Farm-Center suggest using a peroxyacetic acid (PAA)-H_2O_2-mixer during post-harvest WV locally grown produce processing.

Purpose: To I.) evaluate the efficacy of PAA-H_2O_2-mixer to reduce *Listeria monocytogenes* in 0.1% buffered-peptone-water; II.) evaluate the reduction and mitigation of cross-contamination on peppers.

Methods: Nalidixic-acid-resistant (NaL) *L. monocytogenes* were used in this study. For the study-I, aliquots of 1.0-ml the PAA-H_2O_2-mixer solutions (0.25 and 0.50%) were added to the first 3-well of 8-strip-deep-well microplates. Then, the 0.1 ml of 10-fold dilution the bacterial culture was added and mixed immediately with a multichannel pipette. After exposure for 0, 5, 15, 30, to 120-s, the 1 ml of 2×D/E neutralized solution was added to terminate the reaction. Cell counts was determined by spread-plating onto TSA+200 ppm NaL and incubated at 35°C for 48 h. For the study-II, 5 red-peppers were dip-inoculated with *L. monocytogenes* followed by triple-washing (water+water+antimicrobial, 45-s each step) with 15 uninoculated green-peppers treated by the PAA-H_2O_2-mixer at 0, 0.05, 0.1, and 0.18%. Microbial population was determined by an MPN-method. Microbial data were analyzed using the Global-Fit and USDA-Integrated-Predictive-Modeling-Program software and one-way-ANOVA (P=0.05, N=3) in SAS.

Results: Exposing 0.25% and 0.5% of the mixer reduced ($P < 0.05$) *L. monocytogenes* from 8.11 to 0.48 $\log_{10}$CFU/ml and to <0.3 $\log_{10}$CFU/ml after 90 and 120 s reaction time, respectively. Survival of the pathogen fit the classic linear (RMSE=0.5311-0.8273; R^2=0.9052-0.9494) and Weibull models (RMSE=0.5584-0.7666; R^2=0.9322-0.9521). Inactivation parameters Kmax (linear) and Delta (Weibull) increased from 0.12 to 0.17 and 24.70 to 30.25 when the mixer

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increased from 0.25 to 0.50%. Applying 0.05 to 0.18% of the mixer reduced *L. monocytogenes* by 1.49 to 3.71 log_{10}MPN/g and decreased the cross-contaminated cell counts from 4.17 (water-only control) to 3.03 log_{10}MPN/g (0.10%).

Significance: Future studies are needed to determine the minimum concentrations of PAA-H_2O_2-mixer required to completely mitigate microbial cross-contamination on peppers.

P2-170 Efficacy of Foam Decontamination Combined with Commercial Sanitizers in Removing Natural Contaminants on Tomatoes' Surface

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Introduction: Foam decontamination has not been studied as an alternative to typical dump tanks and flumes for reducing microbial load on tomatoes in packinghouses, despite high water and antimicrobial use, as it is more effective and uses less water and chemicals.

Purpose: To evaluate the efficacy of foam in combination of sodium hypochlorite (NaOCl) or peracetic acid (PAA) against Enterobacteriaceae (EB), aerobic plate count (APC), yeast and mold (Y/M) on the surface of tomatoes.

Methods: Foam was generated using Tween 20 (surfactant) in a combination with NaOCl or PAA to be evaluated against EB, APC, and Y/M on the surface of tomatoes. Four treatments were carried out on tomatoes, including water (W), T20, T20 + NaOCl, and T20 + PAA, after they were randomly chosen from three local markets and treated for 1 and 5 minutes. Samples (n=90) were serially diluted in PBS and plated on 3M petrifilms for EB, APC, and Y/M, and incubated for 24, 48, 72-120 h. All testing was conducted in triplicate using SAS for statistical analyses.

Results: The results showed that all treatments were more effective than water and achieved between 2.65 – 4.45 log10 CFU/tomato for all tested microorganisms at a holding time of 1 minute. T20 + NaOCl showed the highest microbial reduction, reducing EB, APC, and Y/M to 4.45, 3.92, and 3.30 log10 CFU/tomato, respectively. After 5 minutes of treatment, the microbial reduction achieved between 2.40 – 4.38 log10 CFU/tomato of all tested microorganisms. Overall, the highest microbial reduction was shown in the following order: NaOCl > PAA > T20 > water.

Significance: An alternative to dump tanks and flumes for reducing microbial load on tomatoes during postharvest operations could be foam decontamination system, which could achieve a higher level of microbial reduction with less water and sanitizer use and thus lower the costs associated with packing.

P2-171 Simulation of the Risk of Microbial Contamination for Dropped and Drooping Grapefruits and Strawberries with Ink

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Introduction: Dropped and drooping (in contact with ground but still attached to the plant) produce may represent a food safety risk since produce damage or ground contact make them more susceptible to pathogen contamination.

Purpose: The objective of this study was to investigate the risk of contamination associated with drooped and dropped strawberries and grapefruits.

Methods: Two trials (4 fruits per treatment, n=8) for both strawberries and grapefruits (washed but not waxed), were conducted where fruits were drooped or dropped on black ink pads. Strawberries were drooped for 0 (touch), 0.16, 1, and 24 h and dropped through PVC pipe (7.62 cm diameter) from 15.24, 30.48, 60.96, 121.96 cm. Grapefruits were drooped for 0 (touch), 24, 72, 168, 336 h and dropped through PVC pipe (15.24 cm diameter) from 15.24, 30.48, 60.96, 121.92, 182.88 cm. Pictures of each fruit were taken and the percentage of the inked area (PIA; area with risk of microbial contamination) were measured using Image J program.

Results: Grapefruits had a higher PIA (16.9±9.8 to 34.3±12.0% by dropping and 1.8±0.6 to 17.5 ± 2.4% by drooping) than the strawberries (8.5±2.7 to 18.9±10.11% by dropping and 2.8±2.2 to 4.4±1.2% by drooping). Regardless of strawberry drooping time, no statistical difference ($p<0.05$) in PIAs was seen over 24 h. The PIA on drooping grapefruit increased significantly ($p<0.05$) as contact time increased after 72 h. When both fruits were dropped, greater PIA was observed with greater heights; no correlation was found between fruit weight and PIA.

Significance: Evaluating the area of fruits impacted by drooping or dropping is an important first step in understanding the difference in food safety risks between drooped and dropped produce.

P2-172 Comparison of Retrofitted Do It Yourself (DIY) Washing Machine with Commercial Drying Unit USED for Drying Local Fresh Produce

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Introduction: The Center for Disease Control has identified contaminated equipment as one of the five major factors contributing to foodborne illness in food establishments. As a lower-cost alternative, smaller-scale farmers use retrofitted washing machines to dry their fresh produce as an alternative to commercial drying units. The potential microbial risks of these units are not well investigated.

Purpose: The main objective of this project is to compare the microbial risks associated with retrofitted washing machine units against commercial produce drying units used with small-scale operations.

Methods: Speed Queen washing machines were converted solely for the spin process after rewiring and maintaining the centrifugal force for drying fresh produce. Fresh spinach was single-washed with water and inoculated with Listeria innocua at 10^3 and 10^6 CFU/ml, respectively. Spinach was then transferred and dried in the retrofitted washing machines unit and commercial drying unit separately. Three contact surfaces of the machines (loading basket, internal chamber, bottom chamber) were sampled using sterile microbial swabs to enumerate the bacterial transfer by plating on the oxford media.

Results: There was nearly complete microbial recovery from the DIY and commercial units, irrespective of the contact surface and inoculum concentration (3 log CFU/ml at lower inoculum concentration). The bottom chamber had the highest recovery overall and the commercial drying unit (3.7+/-0.54CFU/ml) had a higher microbial recovery than the DIY (2.2+/-0.6CFU/ml). This may be attributed to the product design as the commercial unit has a flat bottom surface versus the DIY unit (edged slant surface), which may make it difficult to drain water and lead to high microbial recovery.

Significance: The results from the experiment signifies that the spread of contamination can occur in both units similarly. Therefore, DIY machines offer affordability and ease of use and do not pose a higher microbial risk than the commercial drying unit investigated in this study.

P2-173 Reduction of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on Lettuce (*Lactuca sativa*) and Cucumber (*Cucumis sativus*) by Hot Water and Vinegar Treatment

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Introduction: Fresh produce foodborne diseases are a rising public health concern as they undermined food biosafety.

Purpose: This study purposed to evaluate the efficiency of combined a hot water (HW) and vinegar treatment in reducing foodborne pathogens in lettuce and cucumbers.

Methods: The produce was 75%-ethanol surface sterilized and inoculated with 8 logCFU fresh culture of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. After 30 min. post-inoculation time, the samples were exposed to HW (45 °C to 65 °C for 10 to 40 s) and vinegar (0% to 5% for 5 min.). Following treatment, surviving bacterial cells were recovered and enumerated via spread plating of sample diluents onto nutrient agar at 37°C for 24 or 48 h.

Results: Exposure of *E. coli* O157:H7 inoculated lettuce and cucumber to HW at 65°C/40 s showed the best reduction at 3.28 and 2.21 log CFU, respectively. Lettuce and cucumber inoculated with *L. monocytogenes* at the same temperature-time combination reduced by 2.29 and 1.45 log CFU, respectively. 5% vinegar/5min treatment reduced *E. coli* O157:H7 inoculated onto cucumber by 4.65 log CFU and by 4.81 log CFU on lettuce. On the other hand, 5% vinegar/5min treatment reduced *L. monocytogenes* by 2.49 log CFU on cucumber and 3.8 log CFU on lettuce. While the combination of the best HW and vinegar treatment conditions reduced *E. coli* and *L. monocytogenes* on cucumber by 4.96 log and 4.21 log CFU, respectively, it achieved 5.67 log CFU and 5.2 log CFU reduction of the pathogens respectively, on lettuce.

Significance: In conclusion, a careful combination of HW and vinegar treatment conditions could improve fresh produce safety.

P2-174 Evaluate a Mixer of Hydrogen Peroxide and Peroxyacetic Acid to Mitigate Microbial Cross-Contamination of *Salmonella* Typhimurium and the Surrogate *Enterococcus faecium* during Triple Washing of Butternut Squash

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❖ ***Developing Scientist Entrant***

Introduction: Recently, butternut squash has been involved in voluntary recalls due to suspected microbial contamination. Therefore, butternut squash can potentially be an under-recognized and under-researched emerging food vehicle for foodborne pathogens.

Purpose: This study evaluated the effectiveness of triple washing with a hydrogen peroxide (H_2O_2)-peroxyacetic acid (PAA) mixer to mitigate microbial cross-contamination of *Salmonella* Typhimurium and the surrogate bacteria *Enterococcus faecium* on butternut squash.

Methods: To produce cross-contamination, one or two butternut squash were dip-inoculated with nalidixic acid-resistant *S*. Typhimurium (4.03 $\log_{10}$MPN/g) or *E. faecium* (4.20 $\log_{10}$MPN/g) and then mixed with 6 ("1:6") or 5 ("2:5") uninoculated fresh clean squash followed by triple-washing for 45 s in water, water, and then the H_2O_2-PAA mixer at doses 0, 0.0064, 0.25, 0.50, and 0.84%. The most-probable-number (MPN) was used to determine microbial population. This study was repeated twice by a 2 × 5 factorial experimental design with 2 different bacteria (*S.* Typhimurium and *E. faecium*) and 5 different concentrations of H_2O_2-PAA mixer (0, 0.0064, 0.25, 0.50, and 0.84%). Data of reduction and cross-contamination were analyzed using the JMP software.

Results: Triple-washing squash with 0.25 to 0.84 ml/dl H_2O_2-PAA mixer resulted in greater ($P<0.05$) reductions of *S*. Typhimurium and *E. faecium* by 2.50 to 3.10 and 2.01 to 3.43 logMPN/g, respectively, than the 0 and 0.0064% treated samples. Applying 0.25 to 0.84% H_2O_2-PAA mixer resulted 1.02 to 1.31 and 0.84 to 1.12 logMPN/g cross-contaminated *S*. Typhimurium cell counts in the "1:6" and "2:5" ratio tests, respectively. *E. faecium* showed similar reduced cell counts and cross-contaminated cell counts in most tested treatments compared to *S*. Typhimurium, indicating it is an appropriate surrogate bacterium for *Salmonella* during post-harvest produce washing challenge studies.

Significance: This study's findings will benefit local governmental agencies as they prepare regulations to mitigate food safety microbial risks associated with locally grown fresh produce.

P2-175 Evaluation of Surface Water Treatment Efficacy Protocol Using Calcium Hypochlorite Against *Salmonella spp.* in Florida Water

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Introduction: Microbial pollution can be a significant threat to surface water and has been implicated as a source of contamination on produce resulting in outbreaks. Growers are under market-driven and regulatory pressure to treat surface water prior to use in contact with produce.

Purpose: The study aimed to evaluate applicability of the FDA's revised water treatment efficacy chemical labeling protocol, using agricultural water.

Methods: Surface water from a West Central Florida Farm pond (98 ml) was inoculated with 1 ml of a ca. 10 log CFU/ml Rifampicin resistant seven strain Salmonella cocktail. Water (99 ml) was equilibrated at 12 or 32°C for 30 minutes. Calcium hypochlorite was ground into powder and mixed with PBDW to create a stock solution (150-200 ppm). Stock chlorine solution (1 ml) was added to 99 ml of water to achieve a 2-4 ppm of free chlorine. Following addition of chlorine, at 1 and 5 min, Salmonella populations were determined by serial dilutions in sodium metabisulfite, plating onto non-selective media with rifampicin, and incubating 35±2°C for 24±2 h. Colonies were counted by hand and expressed as log CFU/ml; student t-tests were performed (n=3).

Results: At 32°C log reductions of <6.2 and <6.5 log CFU/ml, at chlorine concentrations 3 and 3.5 ppm, for 1 and 5 minutes, respectively. At 12°C log reductions of < 6.2 and<6.5 log CFU/ml, at chlorine concentrations 3 and 3.5 ppm, for 1 and 5 minutes, respectively. The greater than 6 log reductions under all treatment conditions were statistically significant. No significant differences were found between 12 and 32°C (P value = 0.967).

Significance: Calcium hypochlorite, at concentrations of 2-4 ppm free chlorine, is an effective surface water treatment to reduce *Salmonella* for growers using ponds in West Central Florida.

P2-176 Efficacies of Treatments with Drytec® and Tsunami®100 in Inactivating *Salmonella enterica* on Alfalfa Seeds and Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: Alfalfa sprouts have been linked to outbreaks of gastrointestinal infections worldwide. Control of *Salmonella*, one of the causative agents, has been a challenge to the sprout industry.

Purpose: This study examined the efficacy of treatments with commercial seed sanitizers, Drytec® and Tsunami® 100, in inactivating *Salmonella enterica* on alfalfa seeds/sprouts.

Methods: Commercial alfalfa seeds (2 g) were exposed to 5 log CFU/ml of four individual *Salmonella* strains (*S*. Baildon, *S*. Cubana, *S*. Stanley, and *S*. Montevideo) at room temperature for 1 h. Inoculated seeds were rinsed with sterile water, and rinsed seeds were treated with Drytec® for 15 min and Tsunami® 100 at room temperature for 30 min. Seeds treated with sterile water for 15 or 30 min served as controls. Sanitized seeds were sprouted on 1%

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water agar at 25°C for seven days in the dark. *Salmonella* populations were determined periodically during sprouting. 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 *Salmonella* inoculation level on alfalfa seeds was 3.29 log CFU/g. Seed treatments with Drytec® and Tsunami®100 reduced the mean *Salmonella* population on alfalfa seeds by 3.02 and 3.22 log CFU/g, respectively. During sprouting, the average population of *Salmonella* increased with time and reached the peak population of 4.78 log CFU/g on Day 5. Overall, treatments with Drytec® and Tsunami®100 reduced the mean populations of *Salmonella* on sprouts by 4.85-4.99 log CFU/g and 4.67-4.78 log CFU/g, respectively compared to the water treatment controls. The average populations of the four strains were significantly different (P<0.05) with *S*. Baildon having the highest and *S*. Stanley having the lowest cell populations on sprouts.

Significance: The study shows that treatments with Drytec® and Tsunami®100 are effective in the control of *Salmonella* growth on alfalfa seeds/sprouts.

P2-177 Efficacies of Ascaroside Treatment in the Control of Enterohemorrhagic *Escherichia coli* on Alfalfa and Fenugreek Seeds and Sprouts

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❖ ***Developing Scientist Entrant***

Introduction: Since traditional seed sanitizers have limited efficacy, novel, natural, and more effective antimicrobial intervention is needed for improving the microbial safety of seeds/sprouts.

Purpose: This study examined the efficacy of treatment with ascaroside, a plant immune booster from plant parasitic nematodes, in the control of enterohemorrhagic *Escherichia coli* (EHEC) on alfalfa and fenugreek seeds/sprouts.

Methods: Sanitized alfalfa and fenugreek seeds were treated with 1 mM or 1 µM ascaroside for 20 min. Treated seeds were mixed with lyophilized EHEC cells in sandy soil (10^4 CFU/g). Inoculated seeds were separated from sandy soil, rinsed with sterile water, and sprouted on 1% water agar, with untreated control groups, at 25°C in the dark. On days 0, 1, 3, 5, and 7, seed/sprout samples were collected and analyzed for the populations of EHEC. Data was fit into the general linear model and analyzed using ANOVA. Fisher's least significant test was used to separate the means at a 95% interval.

Results: Results of statistical analyses show that sprout seed type, ascaroside treatment, and sprouting time were the main effects ($P \leq 0.05$) influencing EHEC cell populations on seeds/sprouts. A higher cell population was found on fenugreek than on alfalfa sprouts. The groups treated with both concentrations of ascaroside had significantly lower cell populations than the untreated control groups. The lowest cell population was found in the group treated with 1 mM ascaroside, which, on average, was 3.30 log CFU/g lower than its control group. The group treated with 1 µM ascaroside had a 1.56 log CFU/g lower cell population than the control group. EHEC cell population increased significantly during the first day of sprouting, but the population plateaued between day 3 and day 5 before dropping slightly on day 7.

Significance: The study provides supporting evidence for the possible use of ascaroside treatment to improve the microbial safety of seeds/sprouts.

P2-178 Fate of Foodborne Pathogens on Lemons after Lab- and Pilot-Scale Finishing Wax Application

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Introduction: After washing, lemons are waxed and dried at ambient temperatures to 60°C to extend shelf life. However, data on the impact of finishing wax application on the microbial safety of lemons are limited.

Purpose: The objective of this study was to determine the bactericidal effects of applying finishing wax to lemons followed by a drying step.

Methods: Lemons were inoculated with rifampin-resistant cocktails of *Listeria monocytogenes* (LM), *Salmonella,* or *Enterococcus faecium* NRRL 2354 (EF) at 6.0 log CFU/lemon. In the lab trials, four commercial finishing waxes were applied separately to inoculated lemons using an airbrush compressor, and then lemons were held at 22 or 60°C for 4 min. For each of two pilot-scale trials, 30 EF-inoculated lemons and 120 uninoculated lemons were waxed (87 L/h) and dried at ~50°C. The survival and transfer of inoculated bacteria to a subset of 40 uninoculated lemons was determined by standard plating and enrichment methods.

Results: Application of finishing waxes led to reductions of 2.0 to 3.1 and 2.8 to 4.4 log CFU/lemon for LM and 0.5 to 2.6 and 1.7 to 3.7 log CFU/lemon for *Salmonella* after holding at 22 and 60°C, respectively. Significantly ($P < 0.05$) greater reductions were observed for LM than for *Salmonella* for all treatments. Reductions of EF were lower by 0.2 to 0.8 log CFU/lemon compared to *Salmonella* depending on the wax type and temperature. Applying the wax that gave the greatest reductions under laboratory conditions led to reductions of 1.6 to 2.7 log CFU/lemon of EF after pilot-scale waxing and drying. EF was recovered from 72.5% (29 of 40) of uninoculated lemons by whole-fruit enrichment but not by plating (<1.3 log CFU/lemon).

Significance: Application of finishing wax and a heated drying step can reduce pathogens on lemon surfaces but may also lead to low-level cross contamination when initial contamination is high.

P2-179 Evaluate the Survival of *Listeria monocytogenes* on Organic Honey Crisp and Fuji Apples Stored at 5, 12 and 22.5°C

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Developing Scientist Entrant

Introduction: Contamination of *Listeria monocytogenes* can occur on apples from harvest, to packing and during production supply chain, due to the pathogen's capability of growing in a wide range of temperatures (0 to 45°C), adherence to surfaces, and resistance to antimicrobial disinfectants.

Purpose: To evaluate the survival kinetics of *Listeria monocytogenes* on organic Honey Crisp (HC) and Fuji (FJ) apples during storage at various temperatures.

Methods: Fresh organic HC and FJ apples (without waxing coating) obtained from a local wholesale market were inoculated with a two-strain mix of *L. monocytogenes* followed by storing at 5.0 [22.9% Relative Humidity (RH)], 12.0 (37.0% RH), and 22.5°C (50.4% RH) for 60, 35, and 7 days, respectively, and periodically (day 0 to 60) analyzing microbial populations. Surviving *L. monocytogenes* were spread-plated on Modified-Oxford agar after 10 or 100-fold serial dilutions. Data was analyzed using the mixed-model-procedure of SAS and GinaFit software.

Results: The initial populations of *L. monocytogenes* on HC and FJ apples were 6.23 to 6.89 logCFU/apple for storage at 5, 12, and 22.5°C. The pathogen survival cell counts decreased ($P < 0.05$) to 2.34 to 4.05, 2.72 to 2.98, and 2.47 to 3.75 logCFU/apple by the end of the storage at 5, 12, and 22.5°C. *L. monocytogenes* was more vulnerable ($P < 0.05$) on FJ than HC apples and at room temperature than cold storage temperatures. The inactivation parameters calculated from the Linear, Weibull, and Biphasic models generally are consistent with the pathogen survival curves with few exceptions.

Significance: Results of this study filled the data gap for understanding of microbiological risks associated with postharvest practices of tree fruit production. Future studies are needed to quantify the natural wax amount on various organic apples and develop pre- and postharvest intervention strategies for inactivation of foodborne pathogens on apples as well as other tree fruits.

P2-180 Environmental Monitoring of *Listeria* spp. in Controlled Atmosphere Apple Storage Facilities

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Introduction: Since the infamous caramel apple outbreak of 2014 that sickened 36 individuals with listeriosis, research regarding the relationship between apples and *Listeria monocytogenes* has increased. Previous studies have suggested *Listeria spp.* can be introduced onto apples in apple packinghouses via workers and equipment, subsequently spread throughout the processing environment, and persist even after attempts of thorough cleaning and sanitization. Prior to packing, apples may be held for months in controlled atmosphere (CA) storage rooms. There is a need to investigate if *Listeria spp.* are present in CA storage rooms throughout the storage season.

Purpose: Determine prevalence of *Listeria spp.* in CA storage facilities over two years

Methods: For the 2021-2022 season, 3 CA rooms each at 2 storage facilities were sampled. For the 2022-2023 season, 3 CA rooms each at 3 storage facilities were sampled. 10 zone 3 locations in each CA room were swabbed. Samples were stored at 4°C after sampling and processed within 24 hours. Swabs were enriched in Less-Plus media at a 1:10 w/v ratio and incubated at 37°C for 18-20 hours. Following enrichment, samples were evaluated for the presence of *Listeria spp* using the *Listeria* ANSR assay. Presumptive positives were confirmed by plating onto Neogen *Listeria* Chromogenic Harlequin media.

Results: During the 21-22 storage season, 2 CA rooms at two separate storage facilities had a 15% (9/60) and 18% (11/60) positive rate of *Listeria spp*. for the pre-storage and post-storage sampling period, respectively. During the 2022 pre-storage season, 4 CA rooms across 3 storage facilities had a 10% (9/90) positive rate of *Listeria spp.*

Significance: These data will contribute to a QMRA that will inform apple growers and processors of risks associated with *Listeria monocytogenes* in CA rooms, allowing growers to improve apple handling practices and weigh the benefits of sourcing capital to improve infrastructure.

P2-181 Epiphytic and Internalized Fractions of *Escherichia coli* on Inoculated Live Lettuce Plants and Harvested Leaves

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Introduction: There is substantial variability in the methods adopted for *in situ* inoculation and retrieval of food-borne pathogens presenting challenges when comparing studies.

Purpose*:* We determined the fractions of loosely and strongly attached, and internalized *E. coli* O157:H7 EDL 933 inoculated on Romaine plants (4-weeks old) and store-bought Romaine leaves, using four processing methods.

Methods*:* Two leaves per plant (n=32) or store-bought baby Romaine leaves (n=15) were spot inoculated on the abaxial side with ~6 log CFU per leaf with rifampicin-resistant *E. coli* and incubated for 24 h at 18°C and 65% RH. Leaves were processed in Whirl-Pak bags as *'All'*- homogenized in 2 mL 0.1% peptone water (PW), '*Epiphytic'*- washed in 10 mL 0.1% PW, *'Strongly Attached+Internal'*- washed then homogenized in 2 mL 0.1% PW, and *'Internal'* - washed, sterilized (2% bleach+0.5.% Tween), rinsed in water then homogenized. *E. coli* was enumerated by serial dilution and plate counting.

Results: Lower *E. coli* counts were retrieved from live plants compared to store-bought leaves for each retrieval method (P=<0.05). *Epiphytic E. coli* counts were significantly different than inoculum levels on live plants (1.2 Log lower) compared to store-bought leaves (0.3 Log higher) (P<0.01). No significant differences were found between *All, Epiphytic,* or *Strongly Attached+Internal* Ec fractions from live plants. However, differences in *E. coli* counts were detected in store-bought leaves between *All* or *Epiphytic* and *Strongly Attached+Internal* (P<0.05). *Internal E. coli* retrieved from live plants and store-bought leaves were 7 and 2.6 times lower than *Epiphytic E. coli*, respectively with lower *Internal E. coli* recovered from live plants than store-bought leaves (P<0.01).

Significance: Data suggest that live plants were less supportive of *E. coli* compared to cut leaves, and more resistant to internalization. Retrieval methods utilizing a homogenization step may influence cell viability, underestimating enumeration results. Future studies should select the appropriate retrieval method based on the cell fraction of interest.

P2-182 Comparison of the Recovery Efficiency of Epiphytically Associated *Escherichia coli* O157:H7 on Lettuce Plants Using Different Sample Preparation Methods

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Introduction: Methods used to inoculate, retrieve and enumerate *Escherichia coli* O157:H7 cells on live plants to evaluate lettuce-bacterial interactions vary, confounding comparability of different studies.

Purpose: To compare the efficiency of different processing methods in recovery and quantification of epiphytically associated *E. coli* O157:H7 from inoculated lettuce leaves.

Methods: Romaine lettuce 'Rio Bravo' or 'Outredgeous' were grown for four weeks under a 12-h photoperiod at 22-24°C. True leaves (3rd to 6th) (n=4) were spot-inoculated on either side with rifampicin-adapted *E. coli* O157:H7 strains EDL933 or 2705C (2019 lettuce outbreak strain) at ~6 logCFU/leaf. After 24- and 48-hour incubations at room temperature, inoculated leaves were transferred to peptone water for cell retrieval via stomaching for 5 min (recover epiphytes) or manual-grinding (recover epiphytes + internalized cells) for plate counting. Data were analyzed with multiple regression analysis.

Results: No differences in cell counts (<0.1 log CFU/leaf) were detected between sample processing methods for *E. coli* EDL933 or 2705C (P>0.05). However, significant differences were attributed to the factors of cultivar, strain, incubation duration and leaf side inoculated. *E. coli* EDL933 on 'Rio Bravo' had higher counts (5.5 logCFU/leaf) than 2705C and either strain on 'Outredgeous' (~4.4 logCFU/leaf; p<0.001). Overall, more cells were recovered after a 24-hour incubation, versus 48 hours (p<0.001), with a 0.9 logCFU/leaf difference between 'Rio Bravo' at 24 h and 'Outredgeous' at 48 h (p<0.001). Overall, leaves inoculated on the abaxial side yielded higher counts than adaxially inoculated leaves (p<0.05), with a 1.6 logCFU/leaf difference between abaxial EDL933 and adaxial 2705C on 'Rio Bravo' (p<0.001).

Significance: Although the grinding process might have released internalized bacteria, the two methods yielded similar results, suggesting that stomaching yields a representative estimate of associated *E. coli* O157:H7 on live lettuce plants. However, cultivar, strain, incubation time and leaf inoculation method need to be carefully selected to optimize experimental designs.

P2-183 Comparing *Escherichia coli* O157:H7 Cell Count Recovery from Inoculated Store-Bought Lettuce Using Sonication or Stomaching

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Introduction: Bacterial interactions with lettuce leaves change when plants are harvested and leaves are processed. There is a need for accurate quantification of *Escherichia coli* O157:H7 on harvested leaves in studies investigating interactions between enteric pathogens and leafy greens. Different recovery methods are used in studies investigating *E. coli* O157:H7 on store-bought lettuce, hampering comparability. Time past harvest, however, affects leaf integrity and some methods, such as stomaching can cause leaf disintegration during processing, possibly impacting bacterial quantification results.

Purpose: To compare sonication and stomaching in quantifying epiphytically associated *E. coli* O157:H7 on inoculated store-bought lettuce leaves.

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Methods: Store-bought baby Romaine lettuce leaves (n=3) were spot-inoculated on the adaxial side of leaves with rifampicin-adapted *E. coli* O157:H7 strains EDL933, 2705C (2019 lettuce outbreak strain) or EC4063 (2006 spinach outbreak strain) at ~6 log CFU/leaf. After a 24-hour incubation at room temperature, inoculated leaves were transferred to peptone water for cell retrieval via sonication at 40 kHz for 2 min or stomaching at 250 rpm for 5 min, then plated on tryptic soy agar with rifampicin. The results were obtained via plate-counting and analyzed with multiple regression analysis.

Results: No differences in bacterial counts could be attributed to sample processing methods. Recovery via sonication or stomaching yielded 4.6±0.8 and 4.8±1.0 log CFU/leaf of *E. coli* EDL933, 5.0±1.2 and 5.3±1.1 log CFU/leaf of *E. coli* 2705C, and 4.9±1.1 and 5.2±1.0 log CFU/leaf of *E. coli* EC4063, respectively ($P>0.05$). There was no difference among the counts of the three individual strains ($P>0.05$).

Significance: The similar results yielded by the two sample processing methods suggested that they have comparable efficiency to recover epiphytic bacteria from inoculated lettuce leaves, when they were inoculated with 6 Log of bacteria. These findings will be helpful to optimize experimental designs by developing the least disruptive method without sacrificing the recovery rate.

P2-184 Fate of Viable but Non-Culturable *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Typhimurium on Field-Grown Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* O157:H7 and *Salmonella enterica* Typhimurium are the leading causes of foodborne outbreaks linked to fresh produce. Both species can enter a viable but non-culturable (VBNC) state that can preclude conventional culture-based detection and resuscitate to regain virulence, becoming an increasing concern for food industries. The fate of VBNC *E. coli* O157:H7 and *S.* Typhimurium on leafy greens has not been investigated under field conditions.

Purpose: This study aimed to assess the formation and survival of VBNC *E. coli* O157:H7 and *S.* Typhimurium on field-grown romaine lettuce during pre-harvest and post-processing stages.

Methods: Culturable and VBNC *E. coli* O157:H7 and *S.* Typhimurium were separately inoculated on Romaine lettuce grown in the field plots. The culturable cells were monitored using the plating assay and viable cells were quantified using propidium monoazide (PMA) coupled with qPCR. Romaine lettuce heads were collected from the field and washed with 100 ppm of chlorine, followed by storing at 4°C and 12°C to mimic the commercial post-harvest processing and storage conditions. The presence of *E. coli* O157:H7 and *S.* Typhimurium on the lettuce were tested during the storage.

Results: Viable cell counts of *E. coli* O157:H7 and *S.* Typhimurium were both significantly higher than the culturable cells during 7- to 21-day post-inoculation, indicating the formation of VBNC cells on field-grown lettuce. The concentration of inoculated VBNC *S.* Typhimurium remained relatively stable while VBNC *E. coli* O157:H7 declined to <1 CFU/ml after 21-day post-inoculation. Washing with chlorinated water reduced the detection rate of *E. coli* O157:H7 and *S.* Typhimurium on lettuce after 7-day at both storage temperatures.

Significance: This study enables a better understanding of the fate of VBNC pathogens on the fresh produce during production, post-harvest processing and storage, providing useful information to enhance the safety of fresh produce.

P2-185 Developing Double-Sided Polydimethylsiloxane Artificial Leafy-Greens Phylloplane for Microbe-Plant Interaction Studies

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❖ ***Developing Scientist Entrant***

Introduction: Using real leaf tissues in research often result in large variations and "noise" in results due to the spatial and temporal variations in the leaf properties.

Purpose: We developed double-sided artificial phylloplane for leafy greens (three lettuce varieties, spinach, collard, kale, cabbage) using polydimethylsiloxane (PDMS) and a vinyl-terminated polyethylene glycol chain-based hydrophobicity modifier (PEG) and showed their application in bacteria-plant interaction studies.

Methods: The double-sided molds were casted using PDMS and mature leafy green leaves. The inhibition effect of PDMS-PEG (0-2%) towards *Escherichia coli* O157:H7 87-23 was examined using growth curve in TSB at 37 °C. To make double-sided artificial phylloplane, PDMS and PDMS-PEG (0-2%) was homogenized, degassed, and cured in the mold at 100 °C. The artificial phylloplanes were evaluated for surface morphology, roughness, hydrophobicity, *E. coli* O157:H7 87-23 attachment, and compared with their real leaves. Natural epicuticular waxes from real collard and kale leaves were deposited on their artificial phylloplane. The applications of artificial phylloplane in 1) epicuticular wax effects on the surface properties and bacterial attachment, 2) bacterial growth on the artificial leaves, and 3) sanitizer formulation screening was studied.

Results: PDMS-PEG (0-2%) did not inhibit *E. coli* growth. The double-sided artificial phylloplane were good replicas of real leaves' morphology, thickness, surface microstructure, hydrophobicity, and *E. coli* O157:H7 attachment. By depositing the natural epicuticular wax, artificial leaf showed decreased wetting property ($p < 0.05$), but the wax microstructure enhanced *E. coli* retention ($P< 0.05$). *E. coli* O157:H7 population had similar trends to real lettuce leaves when the artificial lettuce phylloplane was supplied with diluted lettuce lysate (25% at 20 °C and 100% at 4 °C). The artificial lettuce phylloplane had similar behavior to real leaves in the sanitizer screening.

Significance: The double-sided artificial leafy green phylloplane provides a useful tool for future phylloplane microbiology studies and new sanitizer development.

P2-186 Impact of Ultrasound and Malic Acid Combined Seed Sanitation on Broccoli and Radish Microgreen Fungal and Bacterial Microbiomes

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❖ ***Developing Scientist Entrant***

Introduction: Microgreen seeds are the primary source of microbiological contamination. Seed treatments should eliminate hazards without suppressing seedling development and the environment.

Purpose: We developed an environmentally friendly ultrasound-assisted–malic acid (US-MA) seed treatment to inactivate gram-positive and gram-negative bacteria and investigated the treatment impacts on microgreen microbiomes.

Methods: Radish and broccoli seeds were inoculated with *E. coli* O157: H7 87-23 and *L. innocua* ATCC33090 at 3~4 log CFU/g. The inoculated seeds were treated with 5 % malic acid solution with ultrasonication for 5 min. Untreated seeds were used as a control, and efficacy of US-MA treatment was compared to that of 20,000 ppm sodium hypochlorite treatment. The treated and untreated seeds were sprouted in the coconut coir into microgreens at 21 ºC with light exposure (16h/day). The microgreens were harvested on the 8th (radish) or 11th day (broccoli). The bacterial population was enumerated after seed treatment and in microgreens by plate count. The microbial DNA was extracted and sequenced for the 16S rRNA V3-V5 region and the ITS1F-ITS2 for the bacterial and fungal microbiomes. The experiment was performed in triplicates.

Results: US-MA method showed higher effectiveness in reducing *E. coli* and *L. innocua* from radish seeds than the 20,000-ppm chlorine treatment ($P<0.05$). Inoculation of *E. coli* and *L. innocua* altered the bacterial and fungal core microbiome structures of microgreens and their roots. For radish microgreens, the US-MA

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treatment significantly increased the fungal community alpha diversity but lowered the bacterial community diversity ($P<0.05$). However, the US-MA treatment showed opposite effects on the microbiomes of broccoli microgreens. The US-MA and chlorine treatments enriched *Rohodotorula toruloides* percentage and inhibited the genus of Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium in both radish and broccoli microgreens.

Significance: The new US-MA treatment is an effective seed sanitation method, and this study provided insight into treatment impact on plant microbiome.

P2-187 Quantifying Physiological Profiles of Shiga Toxin-Producing *E. coli* O157:H7 during Post-Harvest Pre-Processing Stages of Romaine Lettuce Production

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❖ ***Developing Scientist Entrant***

Introduction: If leafy greens are contaminated in the field, pathogens like STEC O157:H7 can survive throughout the distribution chain. Its survival on pre-harvest lettuce and post-harvest washing has been quantified, but not during harvest and transport prior to processing. Lettuce transported across the U.S. may be held for days at refrigeration temperature prior to processing.

Purpose: Determine if the length of time and temperature changes that occur between harvest and processing impact E. coli O157 physiology and lead to changes in tolerance to subsequent stressors.

Methods: Romaine lettuce harvest temperature data for 2016-2019 was provided by an industry partner and 17°C, representing the 75th percentile of the distribution, was selected for experiments. Greenhouse-grown lettuce was spray-inoculated with STEC O157:H7, harvested after 24h, and held at harvest temperature for 4h before cooling and storage at 2°C. Lettuce samples to quantify culturable, injured, and persister cells, and sanitizer tolerance were taken at the time of harvest, 4h post-harvest, and every 24h after cooling for 5d. Sanitizer tolerance was determined by exposing inoculated lettuce to a 20-27 ppm free chlorine solution for 2min.

Results: Throughout the experiment, culturable cells decreased 0.72±0.34 log CFU/g, and injured cells increased from 1.07±0.99% to 8.13±4.97%. The proportion of cells in the persister state varied over time, starting at 0.23% on the day of inoculation to 23.51% after 4d at 2°C. Chlorine tolerance increased over time, with an average reduction of 0.18 log±0.23 CFU/g after 5 days at 2°C compared to an average reduction of 1.12±0.25 log CFU/g on the day of harvest.

Significance: STEC O157:H7 physiology changes significantly on lettuce during cold storage. These data will be included in a QMRA-based tool for producers to aid in identifying handling practices that decrease risk of STEC transmission via lettuce, while accounting for changes in STEC physiology.

P2-188 Effect of UV-C Light Treatment Against *Listeria monocytogenes* Attached on Fertilizer Contact Surfaces in Hydroponic System

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❖ ***Developing Scientist Entrant***

Introduction: The attachment and biofilm formation of *Listeria monocytogenes* is a concern in the hydroponic production system. Pathogens persists in food production environment through biofilms and are difficult to eradicate through normal cleaning and disinfection.

Purpose: This study examined the attachment of *Listeria monocytogenes* on fertilizer contact surfaces used in hydroponic system and the effect of UV-C light exposure on the levels of *Listeria*.

Methods: Coupons (1 cm²) of materials used in hydroponic system such as PVC pipe, vinyl hose, and high-density polyethylene (HDPE) where spot inoculated (5uL) with *Listeria monocytogenes* at 6.30 Log CFU/cm². The inoculated coupons after drying were dipped in 2 mL of hydroponic fertilizer (pH 6 ± 0.10) solution in a well cell plate. Distilled water was used as the control. The well plates were stored at 25°C, and the coupons were examined on days 1, 3, and 7 for *Listeria* levels. On day 7, the coupons were exposed to UV-C light (3.78 ± 0.71mW-s/cm2) for 1 minute.

Results: A significant ($P < 0.05$) reduction of *Listeria monocytogenes* was observed on PVC and HDPE coupons with reduction of 1.35±0.17 and 0.99±0.16 Log CFU/cm² respectively. *Listeria* levels remained similar in vinyl surface throughout the sampling period. The UV-C light treatment has significant ($P < 0.05$) effect on *Listeria monocytogenes* levels on HDPE and vinyl coupons with reduction of 1.60±0.23 and 1.60±0.83 Log CFU/cm² respectively. No significant ($P < 0.05$) effect was observed on PVC after the UV-C treatment.

Significance: UV-C light treatment could be a potential disinfection technique to mitigate microbial risk in hydroponic systems.

P2-189 Determining the Efficacy of Power Ultrasound Combined with Organic Acid Treatment for the Reduction of Foodborne Pathogens on Romaine Lettuce

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❖ ***Developing Scientist Entrant***

Introduction: Fresh produce, including leafy greens, are frequently linked to foodborne outbreaks. This study evaluated the efficacy of power ultrasound combined with organic acid treatment to reduce *Salmonella enterica* on romaine lettuce.

Purpose: To determine the efficacy of power ultrasound technology incorporated with citric, lactic, and malic acids to reduce the population of *Salmonella enterica* on romaine lettuce.

Methods: Romaine lettuce (25 g samples) was inoculated with a four-strain cocktail of *S. enterica* at 7 log CFU/g. Lettuce was dried for 1 h, followed by treatment for 2 min with 2 or 5% citric, lactic, or malic acid with or without power ultrasound at 40 kHz. *S. enterica* on lettuce was enumerated before and after treatment. Three independent trials with triplicate samples were performed for each condition. Population differences were evaluated via Student's t-test; $P<0.05$ was considered significant.

Results: The initial inoculation level of *S. enterica* on lettuce was 7.00±0.27 log CFU/g. *S. enterica* was significantly reduced on lettuce under all conditions examined. Citric, lactic, and malic acids at 5% resulted in population reductions of 1.49, 3.04, and 2.90 log CFU/g, respectively. The 5% acids coupled with ultrasound treatment resulted in reductions of 1.74, 4.28 and 2.89 log CFU/g, respectively. The addition of ultrasound significantly improved the effectiveness of lactic acid at 5% to reduce the population of *S. enterica* on lettuce; no improvement was observed with citric or malic acids.

Significance: The results of this research indicate that power ultrasound coupled with organic acid treatment may be effective at reducing *S. enterica* on lettuce. This study fills a data gap on the use of this non-thermal processing technology in a combination treatment for fresh produce.

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P2-190 Impacts of Low-Dose Continuous Gaseous Ozone on Fates of *Listeria innocua* on Cosmic Crisp Apples during Commercial Storage

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Introduction: Apples are typically stored for up to 12 months in refrigerated air (RA) or controlled atmosphere (CA) cold storage. Ozone is a strong oxidant and potent antimicrobial agent without leaving any residues.

Purpose: To investigate the effects of six months of low-dose continuous gaseous ozone application on controlling *Listeria innocua* on Cosmic Crisp apples and quality attributes of fruit during long-term commercial cold storage.

Methods: The inoculated or uninoculated Cosmic Crisp apples were subjected to RA or CA with or without gaseous ozone for up to 36 weeks. Apples of ozone groups were treated low dose of continuous gaseous for 24 weeks of CA storage, then followed by 12 weeks of regular CA storage. Apples were samples at 3, 6, 12, 24, and 36 weeks of storage for *Listeria* and resident microflora enumeration.

Results: A 1.6 to 1.7 log_{10} CFU/apple log reduction of *L. innocua* was achieved after three weeks of cold storage when gaseous ozone reached target concentrations. There was 3.4 to 3.5 log_{10} CFU/apple reduction of *L. innocua* on WA 38 apples under RA or CA storage with or without 1-MCP treatment during 36 weeks of storage. A 24-week continuous low-dose gaseous ozone application in CA storage caused an additional 2.4 to 2.8 log CFU/apple reduction. Low-dose gaseous ozone application also retarded the growth of decay microorganisms (0.5 to 0.6) increase vs 0.2 to 0.4 decrease) and resident bacteria (0.6 to 0.7 increase vs 0.2 to 0.4 decrease) on fresh apples. Gaseous ozone application during 9-month storage improved the visual appearance of apples, did not cause ozone burn, and had no impact on the superficial scale, lenticel decay, russet, or CO_2 damage on apples compared to CA alone fruits.

Significance: A 24 weeks of low-dose ozone gas application during CA storage is a promising strategy to control *Listeria* spp. and microbial decay on Cosmic Crisp apples.

P2-191 Effects of Pulsed Light Treatment on Inactivation of *Salmonella* in Packaged Tomato, Microbial Loads, and Quality

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Introduction: Microbial safety of produce like tomatoes and leafy greens continues to be a major concern. Since post-processing contamination is one major contributing factor to foodborne illness, it is important that treatments are applied after packaging. However, once packaged, treatment options are very limited. Pulsed light (PL) is uniquely suited in this situation since it is a non-aqueous, touch-free decontamination option that is capable of penetrating food packaging enclosures.

Purpose: The purpose of this study was to investigate the efficacy of high intensity short time PL application on inactivation of *Salmonella* in packaged cherry tomato as model food.

Methods: Tomatoes were spot inoculated on stem scars. A bacterial cocktail containing three outbreaks strains of *Salmonella* was used as inoculum. Tomatoes were packaged in Polyethylene (PE) films of 25.4, 50.8 and 76.2 µm thickness. Both packaged and unpackaged inoculated tomatoes were treated with PL for up to 60 s. Treated and untreated tomatoes were stored at 10 °C for 14 days. Samples were analyzed for surviving population of *Salmonella*, native microbiota and quality. Experiments were conducted independently in triplicate. Data analyzed using ANOVA and Duncan's LSD method.

Results: A 10 s (10.5 J/cm^2) PL treatment was optimal. Direct treatment for 10 s provided 1.9 ± 0.17 log CFU/g reduction of *Salmonella*. For packaged tomatoes, log reductions decreased with increasing film thickness but not significantly ($P>0.05$). Also, no significant difference in decontamination efficacy between packaged and unpackaged tomatoes was observed. Treatment significantly reduced native microbiota by >1 log. Fruit firmness and visual appearance were not significantly affected by the treatment.

Significance: These data suggest that 10 s high intensity PL treatment may be used to enhance microbial safety and reduce post processing contamination of packaged cherry tomato in the supply chain.

P2-192 Using GFP-Tagged *E. coli* O157:H7 to Evaluate Microgreen Safety from Contaminated Seeds

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Introduction: Microgreens, such as basil, thyme, parsley, and cilantro are gaining popularity for their rich color, flavor, texture, and nutrition. Unlike sprouts, microgreens have not been implicated in foodborne disease outbreaks; however, similarities in production and environmental conditions have raised interest in examining the impact of contaminated seeds on microgreen safety.

Purpose: This study examined the effect of contaminated seeds on microgreen safety using green fluorescent protein (GFP)-tagged *E. coli* O157: H7.

Methods: Three *E. coli* O157: H7 strains were transformed using $CaCl_2$ heat-shock transformation method and tagged with GFP encoding and ampicillin-resistant pGFPuv plasmid. Seeds for thyme, parsley, cilantro, and basil (10gm each) were inoculated with a cocktail of transformed *E. coli* O157:H7 culture to 3 – 3.63 log CFU/g. Inoculated seeds were evenly sown onto the seedling mix on plastic trays and placed in a growth chamber at 24°C (day) and 22°C (night) with 12 h photoperiods and 65% RH. Germinated seeds, microgreens, and roots were harvested over a period of 3 to 11 days and examined for the levels of *E. coli* O157:H7.

Results: The contamination level of GFP-tagged *E. coli* O157:H7 on seeds (3 – 3.63 log CFU/g) increased significantly ($P<0.05$) after germination to 7.17 – 7.54 log CFU/g, however, levels remained similar till the formation of microgreens (7.04 - 7.92 log CFU/g). *E. coli* O157:H7 levels after germination of parsley seeds were significantly higher ($P<0.05$) than basil and cilantro germinated seeds. Levels were similar between parsley and thyme germinated seeds while basil, cilantro, and thyme germinated seeds had similar levels of *E. coli* O157:H7. For all seeds, *E. coli* O157:H7 levels were significantly higher in the roots (7.95 - 8.46 log CFU/g) as compared to the germinated seeds and microgreens.

Significance: Microgreen production provides favorable conditions for the growth of *E. coli* O157:H7 highlighting the risk associated with contaminated seeds.

P2-193 Production of Preservatives in Dried Pollack

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Introduction: Abuse of preservatives in food could be a problem but certain preservatives are produced naturally. Thus, the production can be considered an abuse of the preservative.

Purpose: The purpose of this study was to evaluate the amount of natural preservatives produced during the manufacturing process of dried pollack according to the difference in the freshness of pollack.

Methods: Dried pollack was manufactured with fresh pollack and non-fresh, which was stored at room temperature to decrease freshness. The production of propionic acid, benzoic acid, and sorbic acid in the dried pollack was detected. preservatives Propionic acid was detected by gas chromatography with flame ionization detector and benzoic acid and sorbic acid were detected by high-performance liquid chromatography with photodiode array.

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Results: Propionic acid was not detected in the dried pollack manufactured with fresh pollack during manufacturing, but it was detected in the sample manufactured with non-fresh pollack. The concentration of propionic acid was increased in the dried pollack prepared with non-fresh pollack after the drying process. During the process of manufacturing dried pollack, benzoic A and sorbic acid were not detected in dried pollack samples, regardless of freshness of the pollack.

Significance: These results indicated that propionic acid can be produced naturally in the dried pollack when using non-fresh pollack.

P2-194 Natural Production of Preservatives in Dried Filefish during Manufacture and Storage

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Introduction: Even through preservatives are not added to food, the preservatives are often detected in food. Thus, it is sometimes considers of preservative abuse.

Purpose: The objective of this study was to investigate the preservatives are produced naturally in dried filefish and it according to the freshness of the filefish influence the production of preservatives.

Methods: Fresh and non-fresh filefish fillets were marinated and dried. Grilled dried filefish were stored at 25°C, and non-grilled filefish were stored at 4°C. during the the concentrations of propionic acid, benzoic acid, and sorbic acid were measured. Propionic acid was examined using a Gas Chromatography (GC) Flame Ionization Detector (FID), and sorbic and benzoic acids were examined using a High-Performance Liquid Chromatography (HPLC) Photo Diode Array (PDA) detector.

Results: No propionic acid was produced in dried filefish made from fresh filefish. On the other hand, propionic acid was detected as 11.9±20.6 mg/kg of non-fresh filefish, suggesting that propionic acid increased to 126.4±78.2 mg/kg after drying. During storage, propionic acid was not detected in dried non-roasted filefish made by fresh filefish until the 12th month, and 155.2±58.3 mg/kg at the 0th month to 403.8±231.9 mg/kg at the 12th month of propionic acid was detected in dried non-roasted filefish made by non-fresh filefish. in Grill-roasted dried filefish, The concentration of propionic acid was 93.6±91.4 mg/kg at the 5th month and gradually decreased in fresh filefish products, but the concentration of propionic acid increased to 93.6±76.3mg/kg at the 3rd month and gradually decreased in non-fresh filefish products. Sorbic acid and benzoic acid were not found in any of the samples.

Significance: This result suggests that propionic acid occurs naturally and its concentration may change when making and storing dried filefish.

P2-195 Preservatives Produced during the Manufacture of Fish Bone Calcium

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Introduction: Fish bone calcium is a calcium component extracted by hydrolyzing fish bones with enzymes and is used as a food supplement. Naturally occurring preservatives produced in the process of decomposing aquatic products by enzymes are becoming a problem.

Purpose: The purpose of this study was to confirm whether preservatives are produced during the production of fish bone calcium.

Methods: Salmon bones were placed in a 10% acetic acid solution of 10 times the amount of salmon bones and reacted with this mixture added pepsin at 40°C for 12 h to hydrolyze the fish bones. The reaction solution was centrifuged and filtered with a 0.2 μm membrane filter. The filtrate was freeze-dried to produce fish bone calcium. Salmon bones, samples after hydrolysis, samples after filtration and samples after drying was analyzed for propionic acid with gas chromatography-flame ionization detector, and for sorbic acid and benzoic acid with high-performance liquid chromatography-diode array detector.

Results: Fish bone calcium was prepared as same as the commercial product. Propionic acid was not detected in raw salmon bones. However, 8.1±11.1 mg/kg, 17.6±7.7 mg/kg and 50.8±14.0 mg/kg of propionic acid were detected in samples after hydrolysis, after filtration, and after drying, respectively. Sorbic acid and benzoic acid were not detected in all samples.

Significance: The results of this study suggest that propionic acid can be produced naturally in fish born calcium. Thus. It can be scientific evidence for the natural production of propionic acid in fish bone calcium during the manufacturing process.

P2-196 Production of Propionic Acid, Benzoic Acid, and Sorbic Acid in Fish Collagen Production

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Introduction: The consumption of fish collagen is increased. Even though preservatives cannot be used int the fish collagen, preservatives are occasionally detected. Thus, the investigation is necessary to determine if the preservatives are produced during the production of fish collagen.

Purpose: The objective of this study was to investigate if preservatives are produced during fish collagen manufacturing process.

Methods: Five brands of fish collagen were purchased. Fish collagens were manufactured according to the following steps; 1) immersion of fishskin in distilled water at a ratio of 1:10, before placing the fishskin at 55°C for 250 rpm and 30 min, 2) hydrolysis of the fishskin by adding 0.06% alcalase and 0.06% neutrase, 3) first filtration of the hydrolytes with 0.2-μm filter, 4) inactivation of the enzymes by heating at 55°C for 30 min, 5) deodorization with activated carbon, 6) second filtration with 0.8-μm filter, and 7) freeze-drying of the product. Propionic acid, benzoic acid, and sorbic acid of fish collagen were measured by high performance liquid chromatography.

Results: In the commercial fish collagen samples, propionic acid was detected in one sample at 13.1 mg/kg. However, propionic acid, benzoic acid, and sorbic acid were not detected in the fishskin, samples from manufacturing process, and samples after the process.

Significance: These results indicate that propionic acid, benzoic acid, and sorbic acid may not be produced in the manufacturing process of fish collagen. The commercial fish collagen samples that had propionic acid may be produced with non-fresh fishskin.

P2-197 Hypochlorous Acid Applications during the SARS-COV-2 Pandemic

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Introduction: Hypochlorous acid (HOCl) is a sanitizing agent and can be produced through chemical or electrochemical reactions. The main ingredient of electrolyzed water is also hypochlorous acid.

Purpose: This presentation will focus on the applications and efficacy of HOCl against SARS-CoV-2 virus and the limitations and precautions on using hypochlorous acid.

Methods: Our previous research has demonstrated HOCl is effective to inactivate murine norovirus (MNV-1) surrogate for human norovirus and hepatitis A virus (HAV). Extensive literature search was conducted on HOCl treatment on SARS-CoV-2 virus during the pandemic and its applications on food-contact surfaces.

Results: During the SARS-CoV-2 pandemic, HOCl was demonstrated to be an inexpensive, widely available, nontoxic, and practical disinfectant for routine office cleaning. Medical applications like mouth rinse, nasal spray, and medical equipment sterilization were also suggested. WHO recommended a 1,000 mg/L conc. for waste management and ambient cleaning in clinical laboratories for SARS-CoV-2. A 5,000 mg/L conc. for blood and body fluids spills.

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HOCl can achieved greater than 99.8% virucidal efficacy in less than 1 min on SARS-CoV-2. A safety profile test on both nasal and oral mucosa (rabbit model) indicates that HOCl is non-irritant. HOCl at a sufficient volume and concentration can inactivate SARS-CoV-2 virus regardless of pH and manufacturing method. However, many HOCl-based products on the market had inappropriate product information (e.g. no concentration, no pH, no expiration, no correct amount to use) or inappropriate use recommendations (direct spray to people and fogging of indoor space). WHO and US-CDC both stated that HOCl should be used only for products and environment surfaces and is not for external or internal use by humans or for fogging of indoor spaces because the chlorine gas carries a risk of adverse respiratory system event.

Significance: Efficacy of HOCl for treating SARS-CoV-2 virus were confirmed and applications for food contact-surfaces, environmental, and PPE treatments were suggested.

P2-198 Preharvest Mitigation of Norovirus in Agricultural Water Using Chemical Sanitizers

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Introduction: Human norovirus is the leading cause of foodborne illnesses in the United States, and fresh produce has been linked to several outbreaks. There are abundant water treatment options to combat pathogens; however, research data on their anti-noroviral properties in agricultural waters are limited.

Purpose: To assess the antimicrobial efficacy of a commonly used calcium hypochlorite-based sanitizer in irrigation water collected from a Florida farm.

Methods: Water samples, at respective volumes of 100 µL and 100 mL, were inoculated with 5 $\log_{10}$ PFU of Tulane virus (TuV), a cultivable human norovirus surrogate, and 9 $\log_{10}$ CFU of rifampicin-resistant *Escherichia coli* TVS 353, which was used as a bacterial model. Experiments were performed in triplicate for 2-50 ppm free available chlorine (FAC) at 5 and 10 min contact times (12ºC). Following exposure, the residual microbial loads were quantified with plaque assay on LLC-MK2 monolayer cells for TuV and with standard plate count on BHI-Rif agar for *E. coli*.

Results: A 2-4 ppm FAC for 10 min was ineffective against TuV (P>0.05). After 5 min in 20 ppm FAC, a 1.5±0.4 $\log_{10}$ PFU reduction in TuV was observed (P<0.05), followed by a near inactivation (>4.8 $\log_{10}$ PFU) at 50 ppm. Increased contact time to 10 min did not enhance virus inactivation (P>0.05). At 2 ppm FAC, *E. coli was* reduced for 2.9±0.3 $\log_{10}$ CFU regardless of exposure time. Increasing contact time from 5 min to 10 min led to an enhanced antibacterial activity at 10 ppm FAC (or higher) with respective $\log_{10}$ reductions of 4.8±0.3 and 5.5±0.0 (complete inactivation, P<0.05).

Significance: The sanitizer at 2-4 ppm FAC effectively mitigated the bacterial load in preharvest water to an acceptable level of >3-$\log_{10}$ reduction; however, 20-30 ppm FAC was required to achieve a similar virus inactivation, highlighting the need for further validation of sanitizers for agricultural waters.

P2-199 Persistence of Coronavirus on Food Contact Surfaces and Secondary Transfer Efficacy to Artificial Human Skin

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Introduction: The onset of the COVID-19 pandemic and concerns surrounding the risk of SARS-CoV-2 survival on contact surfaces and the secondary transmission to humans have put a strain on the food supply chain. Currently, epidemiological studies have concluded aerosols and droplets to be the primary routes of SARS-CoV-2 transmission; however, the secondary transmission of the virus to humans has remained understudied.

Purpose*:* To examine the persistence of coronavirus on food contact surfaces and determine virus transmission rates to artificial human skin.

Methods*:* 1.5 by 1.5 cm coupons of clamshell takeout containers and coffee cup lids were dry-inoculated with 10 µl of 8 log PFU Phi6 bacteriophage, a surrogate for SARS-CoV-2. Virus persistence was determined over a six-day incubation period at 4°C or 25°C under 45% or 65% relative humidity levels (RH). Transfer rates were examined by placing the artificial skin over the inoculated coupon and applying 250-g force (10.9 kPa) for 10 s with the index fingertip at a 45° fixed-angle. Following elution with LB broth, virus infectious titers were quantified using agar overlay plaque assay.

Results*:* There was a significant reduction in viral titer over time across all conditions for both contact surfaces (p<0.05). However, the difference in viral titers was not always statistically significant. For clamshells, virus decay was highest at 25°C-65% RH, reaching a 4.9 log PFU reduction at day 6 (P<0.05). Data for the cup lid at 25°C-65% RH is still being collected and will allow us to further examine the cumulative effect of temperature and RH on viral reduction. Clamshell and cup lids showed respective mean transfer rates (%) of 1.5 ± 0.5 and 7.2 ± 1.9 to artificial human skins.

Significance*:* The outcomes of this study can help establish risk-based assessment models on the persistence and secondary transmission of coronavirus in the food supply chain.

P2-200 Fungal Composition Change in Compost Due to Compost Types and *Listeria monocytogenes* Intrusion Using 18S rRNA Gene Sequencing Analysis

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Introduction: The presence of *Listeria monocytogenes* can induce the functional composition change of microbiome in animal wastes-based composts. However, there is a knowledge gap on the impact of compost types and *L. monocytogenes* intrusion on the fungal community.

Purpose: The objective of this study was to evaluate the change in fungal dynamics in dairy- and poultry wastes-based composts with or without *L. monocytogenes* by 18S high-throughput sequencing.

Methods: Both dairy- and poultry wastes-based composts (n = 12) were collected, and the indigenous mold and yeast populations were enumerated by Dichloran Rose Bengal Chloramphenicol Agar. To understand the dynamic changes in fungal community, compost samples were inoculated with ca. 7 log CFU/g of *L. monocytogenes*. DNA extraction was performed at 0 and 72 h post-incubation at room temperature with the dead cells being removed by propidium monoazide treatment. The extracted DNAs were subjected to 18S rRNA gene sequencing. High-quality sequenced reads were analyzed using a custom modified QIIME2 analysis pipeline.

Results: The culturable mold and yeast ranged from ca. < 2.1 to 6.3 log CFU/g. *Rhabditida* (22.5%), *Nucletmycea Fungi* (19.5%), *Arachnida Acari (18.7%)*, *Cercomonadidae Cercomonas (10.7%)*, and *Chromulina Chromulina* (7.3%) were found as top-5 dominant fungal genus found in compost samples. Composting stage affected (P < 0.05) alpha diversity, observed species and Shannon diversity index for poultry composts but not dairy composts. Regardless of the composting stage, poultry compost had a significantly (P < 0.05) higher alpha diversity than dairy compost along with different dominant fungal phyla. Furthermore, the fungal compositions of compost samples were separated in PCA by the compost types and stage but not by other experimental factors (moisture contents, incubation time, and *L. monocytogenes* intrusion).

Significance: This study has provided some baseline data on the fungal community in a variety of animal wastes-based composts.

P2-201 Efficacy of Ready-to-Use Spray Disinfectants Against SARS-COV-2 Surrogates, Bovine Coronavirus, and Human Coronavirus OC43 on Surfaces Commonly Found in the Front-of-the-House in Foodservice Establishments

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Introduction: Due to the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a multitude of foodservice establishments (FSEs) have been adversely impacted. Effective environmental decontamination procedures in FSEs are necessary to prevent and control spread of SARS-CoV-2. However, data regarding disinfectant efficacy against SARS-CoV-2 or surrogates on surfaces commonly found in the front-of-the-house in FSEs is limited.

Purpose: The aim of this study was to determine the efficacy of three ready-to-use (RTU) spray disinfectants against two SARS-CoV-2 surrogates, bovine coronavirus (BCoV) and human coronavirus (HCoV) OC43.

Methods: Three RTU disinfectant sprays of different active ingredients (chlorine, hydrogen peroxide, and quaternary ammonium compound + alcohol) were selected from EPA List N. First, a neutralization method was optimized for each tested disinfectant. Then, the efficacy of three disinfectants against BCoV and HCoV OC43 was determined in suspension and on two non-porous surfaces [polyethylene terephthalate (PET) plastic and vinyl upholstery fabric] with a soil load of 5% FBS. Titers of infectious BCoV and HCoV OC43 were quantified by the median tissue culture infectious dose ($TCID_{50}$) assay.

Results: After a 2-min contact time, all three disinfectants achieved a >3.0 log reduction of two surrogates in suspension and reduced virus infectivity on both surfaces below the limit of detection (0.6 log $TCID_{50}$/carrier). Further statistical analysis of carrier test data revealed mean titer reduction of both surrogates after drying was greater ($P<0.05$) on vinyl than PET, and under higher relative humidity (RH) than lower RH on both carriers. At lower RH, the titer reduction of HCoV OC43 was greater ($P<0.05$) than BCoV on both carriers.

Significance: Our results showed both SARS-CoV-2 surrogates are very sensitive to the tested disinfectants. Further analysis indicates additional factors (surface type, RH, and surrogate) must be considered when performing disinfectant efficacy testing on carriers.

P2-202 High Humidity Causes Mutation of the SARS-CoV-2 Surrogate Phi6

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❖ ***Developing Scientist Entrant***

Introduction: The enveloped, double stranded RNA virus Phi 6 has been used as a surrogate for the study of enveloped viruses like SARS-CoV-2. While risk of SARS-CoV-2 transmission from surfaces is thought to be low, viral evolution may change this risk.

Purpose: The purpose of this study was to evaluate the effects of high (100%) relative humidity at 35°C on the viral evolution of bacteriophage Phi 6.

Methods: Five populations of Phi 6 were each subjected to daily passages of 15 minutes at 100% RH, 35°C for a total of 30 days. Phi 6 lysates (10 drops of 1ul at ~ 10^{10} PFU/ml) were applied to stainless steel coupons and sampled through plaque-forming assays using *Pseudomonas syringae* pathovar *phaseolicola* as the host and the top agar overlay technique. Viable viruses were re-cultured and re-inoculated repeatedly onto new coupons over 30 days. Viral RNA was extracted from selected passages and libraries prepared and sequenced. Raw reads were trimmed, filtered and mapped to a phi 6 genome reference sequence.

Results: Phi 6 populations showed decreased tolerance to RH stress up to five passages relative to its initial population (~ 5.1 log reduction). All populations showed increasing tolerance after 5 passages and were eventually highly tolerant (0 to 1 log reduction) to RH stress. Sequencing revealed a total of 163 single nucleotide polymorphisms with 49 of these present in at least two distinct passages. At least two mutations were shared by more than 50% of the populations and were found in two regions of its segmented tripartite genome: one in the 5' untranslated region on the small segment, and one in the gene P6 on the medium segment.

Significance: These results will be useful in understanding and managing the risk of environmentally induced changes in enveloped RNA viruses.

P2-203 Effect of Temperature and Relative Humidity on Survival of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), a Potential SARS-CoV-2 Surrogate on Food Contact Surfaces over Time

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Introduction: The survival of the SARS-CoV-2 virus in the food processing environment depends on the nature of food contact surfaces (FCS) and environmental conditions. Identifying the persistence of PRRSV, a potential SARS-CoV-2 surrogate on different FCS and environmental conditions is critical to the food industry and public health

Purpose: To study the persistence of PRRSV on FCS in simulated food processing environments (temperature and relative humidity (RH)).

Methods: The PRRSV (> 6 logs tissue culture infective dose, TCID50) was inoculated onto polypropylene (PP) and stainless steel (SS) coupons (2.2 in. cm x 2.2 in.) with organic load and allowed to dry for 15 min. followed by incubation at 4 °C/25 °C and 45%/65% RH. On day 0, d1, d3, d5, and d7 post-inoculation, the FCS were processed for virus recovery and titration using the TCID50 method in PRRSV permissible MARC-145 cell line in a 96-well tissue culture plate. The experiment was conducted in triplicate and data were analyzed using one-way ANOVA. Means were separated at a 5% level of significance.

Results: At 4 °C/45% RH and 4 °C/65% RH, infectious PRRSV was recovered (>3.6 and >2.9 logs TCID50 respectively) until d7 from both surfaces. At 4°C/45% RH, the persistence was lower ($P \leq 0.05$) on d3 and d1 on PP and SS respectively, whereas at 4°C/65% RH, the virus titer was constant until day 3 before it lowered. At 25 °C/45% infectious virus was detected until day 3 on PP and until d1 on SS with significantly lower titer on successive recovery. At 25 °C/65% RH, viruses were not detected after d0 on either surface.

Significance: The results indicate that the chances of survival of SARS-CoV-2 and its surrogates are higher on FCS at normal food processing environmental conditions as compared to abused environmental conditions.

P2-204 Survival of the Sars-Cov-2 Surrogate Bacteriophage Phi6 on Food Industry Surfaces across Temperature and Relative Humidity

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❖ ***Developing Scientist Entrant***

Introduction: SARS-CoV-2 has caused great disruption to the food industry and data are needed to understand it's risk of survival and transmission from food contact surfaces. Bacteriophage Phi6 has been proposed as a biosafety level 1 surrogate for SARS-CoV-2 since it also possesses a lipid envelope, membrane spike protein and RNA.

Purpose: The purpose of this study was to determine the survival of bacteriophage Phi6 on relevant contact surfaces in the food industry across various environments as a surrogate for SARS-CoV-2.

Methods: The survival of Phi6 on relevant food industry surfaces (corrugated cardboard, stainless steel, nitrile gloves (VWR), nitrile gloves (Fischer), rubber conveyor belt, take away cups (polyethylene lined) and lids (polystyrene #6), clamshell (polyethylene terephthalate), and laminated food packaging was measured under different conditions. Ten 1ul drops of lysogeny broth containing ~1_E^{+10} plaque forming units of Phi6 were inoculated onto surfaces,

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air dried, then stored at real-world combinations of three temperatures (7, 25 and 37˚C) and three relative humidities (RH) values (45, 65, 85%). Viable Phi6 was evaluated immediately after drying, then over minutes (higher temperature and humidity) and up to 91 days (lower temperature and humidity). Virus was recovered in 10ml lysogeny broth using one minute of the rub shake method. Each experiment was conducted in triplicate.

Results: Temperature and humidity affect the survival of Phi6 across surfaces, with both lower temperature and humidity favoring survival. Retrieval from gloves was poor, while corrugated cardboard had high survival across all conditions (one-log reduction in phage after ~63 days at 7˚C and 50% RH and after ~12 days at 35˚C and 50% RH (R2=0.76 and 0.75 respectively).

Significance: These data increase our understanding of risk posed by enveloped viruses to persist on various food industry surfaces and environments.

P2-205 Survival of Phi6 on Three Clean or Soiled Food Contact Surfaces at Various Temperature and Humidity Conditions

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Introduction: It is established that the primary mode of transmission of SARS-CoV-2 is airborne. Survival of the virus on food contact surfaces has not been widely investigated.

Purpose: The objective is to evaluate the survival of Phi6, a SARS-CoV-2 surrogate on clean and soiled food contact surfaces at 4, 25, and 37°C in combination with 45 and 65%RH.

Methods: Clean or soiled (5% yeast extract, 5% bovine serum albumin, and 4% bovine musin) corrugated cardboard, ribbed rubber, or smooth rubber coupons (3×3 cm) were inoculated with 10µl of phi 6 lysate to achieve 8 log PFU/coupon. Coupons were dried in a biosafety cabinet for 10 minutes and sampled at 0, 1, 3, 24, 48, 120, and 168 h. Coupons were stored at 4, 25 or 37°C in combination with 45 or 65% RH. At each time point coupons were placed in a sterile whirl pak bag containing 10ml of LB medium, gently massaged for 30 seconds followed by shaking for 30 seconds. Samples were serially diluted in LB and survival analyzed by plaque assay. Three trials were completed with duplicate samples (n=6).

Results: The largest declines were seen on all surface types when held at the highest temperature/humidity combination, regardless of coupon cleanliness. Phi6 inoculated onto clean or soiled cardboard declined to ≤2 PFU/ml at 37°C and 65% RH while levels declined on clean and soiled rubber surfaces to ≤ 2log PFU/ml held at 25°C and 65%RH. Reductions ranged from 1.0 Log PFU/coupon (soiled ribbed rubber, 4c 45%RH) to ≥6.3 PFU/ml (clean smooth rubber 25°C and 65%RH).

Significance: Survival on Phi6, a surrogate for SARS-CoV-2, is impacted by temperature and relative humidity. Survival on food contact surfaces is the longest under refrigerated conditions and low relative humidities.

P2-206 Assessment of Two Approaches for the Quantification of Male-Specific Coliphage in Municipal Wastewater

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Introduction: Male-specific coliphages (MSCs) are culturable viruses that are morphologically similar to human enteric viruses and have been suitable surrogates to establish the effectiveness of wastewater treatment facilities in reducing virus levels. These coliphages have also served as reliable indicators of viral contamination in shellfish and produce irrigation waters.

Purpose: This study sought to compare the efficacy of the PEG/ultracentrifugation technique (primarily used to quantify enteric viruses), to double agar overlay (DAL), the standard technique to quantify MSCs in municipal wastewater.

Methods: Raw, untreated municipal wastewater, was collected from two local wastewater treatment facilities (MS and AL). Three 800ml aliquots of this wastewater were concentrated by polyethylene glycol (PEG) precipitation, followed by ultracentrifugation. Wastewater concentrates were evenly distributed into 200µL aliquots, with one aliquot tested for MSCs immediately and another tested after overnight storage at -80°C. MSCs were enumerated using the DAL technique, utilizing *E. coli* F_{amp} host bacteria and a male-specific bacteriophage (MS2) positive control. Untreated wastewater was directly subjected to the DAL. Plaques were enumerated after incubation at 37°C for 18±2h.

Results: MSC levels in wastewater subjected to PEG/ultracentrifugation from the AL and MS facilities were 5.1 and 5.6 $\log_{10}$ PFU/100mL, respectively. In raw, untreated wastewater directly enumerated by the DAL, the MSC levels were 5.3 and 5.6 $\log_{10}$ PFU/100mL for AL and MS, respectively. In the frozen concentrates, MSC levels from the AL and MS facilities were 4.8 and 4.6 $\log_{10}$ PFU/100mL, respectively.

Significance: The DAL and PEG/ultracentrifugation techniques resulted in similar levels of MSC in wastewater. The result demonstrates that PEG/ultracentrifugation can be expanded to the quantification of MSCs, along with its intended quantification of human enteric viruses. Future studies will focus on the efficacy of this approach to quantify MSCs and enteric viruses in treated wastewater, where levels are presumed to be lower.

P2-207 Photodynamic Inactivation of Norovirus Surrogate Bacteriophage MS2 in Fresh Blackberry Using Curcumin as Photosensitizer

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Introduction: Blackberries (*Rubus* sp.) have been linked to acute gastroenteritis outbreaks caused by Norovirus (NoV). Photodynamic treatment (PDT) using blue light-emitting diode (LED) coupled to natural photosensitizers has been suggested as an eco-friendly preservation technology with viricidal effects. Curcumin is a bright yellow phenolic found in *Curcuma longa* plants. The effectiveness of PDT on NoV inactivation in blackberries using curcumin as photosensitizer remains unknown.

Purpose: This research evaluates PDT coupled to curcumin (PDT-curcumin) on inactivation of the NoV surrogate bacteriophage MS2 on fresh blackberry and its effects on fruit color and texture.

Methods: Sanitized blackberries (5 g) were inoculated with MS2 (100 µl; ~8 log PFU/g). Fruits were immersed in curcumin (75 µM) for 2 min and exposed to PDT (LED at 430 to 470 nm wavelength and 33.6 mW/cm^2 of light irradiance) for up to 12 min. MS2 viral titer on blackberries was determined using *Escherichia coli* C3000 as host on tryptic soy agar (TSA) by the double-layer plaque assay before and after 8, 10 and 12 min of exposure. Treatments with only LED and only curcumin exposure (negative controls) were also evaluated. Instrumental color and textural parameters were assessed in blackberries before and after 12 min of exposure to treatments. Statistical significance was considered for $P<0.05$.

Results: MS2 titer decreased significantly by 2.3 and 3.2 log PFU/g after 8 and 10 min of PDT-curcumin exposure with no further decrease after 12 min. MS2 titer did not change significantly in negative controls, regardless of the exposure time. No significant changes were observed in hue angle, saturation, and brightness or hardness, resilience, springiness, gumminess and chewiness of blackberries after PDT-curcumin exposure.

Significance: PDT-curcumin treatment of NoV surrogate MS2 in fresh blackberries is moderately effective and does not compromise important quality parameters in blackberries.

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P2-208 Evolution of *Listeria* Phage LP-125 to Improve Efficacy under Specific Food Conditions

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❖ ***Developing Scientist Entrant***

Introduction: Bacteriophages are viruses that infect and kill bacteria. There are several phage products available in countries as control agents for *Listeria monocytogenes* in food. Previous work has shown that *Listeria* phages can evolve *in vitro* to overcome challenging resistance types.

Purpose: The goal of this study is to (i) determine if evolution of *Listeria* phages can be exploited to improve their efficacy under specific food conditions, (ii) identify phage mutations that are selected under these conditions and (iii) determine how mutations in phages may improve their efficacy under food relevant conditions.

Methods: Ultra-pasteurized oat and whole milk were chosen as a representative test matrix. Ancestral LP-125 was passaged through 10 rounds of infection in each milk and phage growth kinetics were observed for each round of infection. Plaque purified DNA samples from wild-type and milk-evolved phages were sequenced. Evolved phage reads were compared to the ancestral LP-125 phage assembly to identify mutations. Adsorption assays comparing the performance of the mutant phages against ancestral LP-125 phage were conducted in both test matrices with *L. monocytogenes* 10403S as the host. LB MOPs growth media was used as a control. Data of three replicates were analyzed using Tukey's Honestly Significant Difference All Pairwise Comparison test.

Results: In the oat milk-evolved phages, a radical nonsynonymous mutation was identified in the baseplate tail protein and in the whole milk-evolved phages, a radical nonsynonymous mutation in the baseplate spike protein was observed. These genes are both involved in adsorption. When testing adsorption kinetics, the milk-evolved phages adsorbed significantly faster than the ancestral phage ($P<0.05$). This binding phenotype is not seen in LB MOPs growth media indicating it is specific to the milk conditions.

Significance: In vitro evolution may be useful for generating specialized phages for applications under specific food relevant conditions.

P2-209 UV-LED Technology for the Inactivation of Tulane Virus in Apple Juice and Coconut Water

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Introduction: Ultraviolet light (UV-C at 254 nm) has traditionally been used for surface decontamination. User-friendly, portable UV-C light emitting diodes (LED) are being researched for antiviral effects. Human norovirus (HNoV) illness spread from the food environment needs to be controlled. Tulane virus (TV) is used as the HNoV surrogate for inactivation studies.

Purpose: The purpose of this research was to determine the inactivation of TV when treated with UV-C (254 nm) compared to UV-C LED (279 nm) in apple juice (AJ) and coconut water (CW).

Methods: TV (0.5 mL) at ~7 log PFU/ml was aseptically mixed with 4.5 ml AJ or CW in sterile glass beakers and continually stirred during treatments with 254 nm UV-C for 0 to 10 min or 279 nm UV-C LED for 0 to 25 seconds. 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 LLC-MK2 cells within 6-well plates were performed. Plaques were visualized after incubation for 2 to 3 days. Data from three replicate trials were analyzed using mixed model analysis of variance with Tukey's adjustment ($P≤0.05$). The dose (D10) required to inactivate one-log target population was calculated using the linear model.

Results: Significantly lower D10-values for TV with UV-C LED in AJ and CW than with UV-C at 254 nm were obtained ($P≤0.05$). With UV-C LED, D10-values for TV were 1.50±0.22 and 4.26±1.02 mJ/cm^2, while D10-values with UV-C at 254 nm were 3.34±0.91 and 10.21±1.48 mJ/cm^2 in AJ and CW, respectively. After treatments, no visual changes in color or appearance of treated fluids were observed.

Significance: Data showing improved inactivation of TV using UV-C LED in the tested liquids over conventional UV-C at 254 nm aid in the design and implementation of precise UV-C treatments to inactivate HNoVs.

P2-210 Effect of Pulsed Light on Decontamination of Foodborne Viruses in Various Frozen Fruits

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❖ ***Developing Scientist Entrant***

Introduction: The frozen food market is one of the largest segments of the food industry and continues to grow globally. Thus, viral contamination outbreaks by human noroviruses (HuNoV) and hepatitis A virus (HAV) associated with frozen foods consistently pose a public health threat. Frozen fruits, in particular, are major vectors for foodborne viral infections. Between 2008 and 2018, 14,516 cases of HuNoV and 2,114 cases of HAV were officially reported worldwide in association with frozen fruits such as raspberries, strawberries, and pomegranate arils. Therefore, it is necessary to introduce new control strategies, such as pulsed light (PL), to reduce potential outbreaks of foodborne viruses by frozen fruits.

Purpose: In this study, the ability to inactivate foodborne viruses is evaluated by applying PL to various frozen fruits: blueberry, raspberry, strawberry, blackberry, cranberry, cherry, mango, and pineapple.

Methods: Fresh fruits were artificially contaminated with 30µL of murine norovirus 1 (MNV-1) or HAV suspension (10^6 PFU/ml) and then frozen for 48h. PL treatment with 16 pulses (11.52 J/cm^2) was applied. After treatment, the virus was recovered using Earle's Balanced Salt Solution (EBSS). The recovered virus sample was serially diluted, and its titer was assessed by plaque assay in triplicate.

Results: Frozen blueberries, blackberries and strawberries showed more than 2.1 log reduction of MNV-1 after PL treatment, whereas raspberries showed 1.8 log reduction. The difference in titer reduction between berries was insignificant (ANOVA, $p>.05$). Interestingly, even after freezing the fruits, the virus inactivation effect by PL showed a similar pattern (1-2 log reduction) to previous studies using fresh fruits. On the other hand, unlike fresh berries, there was no darkening in frozen berries, and they remained frozen. The rest of the fruits are under investigation.

Significance: The effect of PL on various frozen fruits obtained through this study will potentially provide a new strategy to control such foodborne viruses.

P2-211 Evaluation of Antiviral Activity of Essential Oils and Natural Extracts

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Introduction: Essential oils (EOs) and natural extracts have long been used as medicines, flavouring agents, and food preservatives due to their content of antimicrobial, antifungal, insecticidal, antiparasitic and antioxidant compounds. However, there are few studies on the antiviral potential of these compounds. Enteric viruses are the main cause of foodborne diseases in the world. They are recognized as a major concern for the health care and food sector in Canada. Therefore, there is a need to look for antiviral strategies, such as bioactive compounds to prevent foodborne illnesses.

Purpose: The aim of this project is to study the antiviral potential of two EOs and four natural extracts on non-enveloped viruses: murine norovirus 1 (MNV-1) and hepatitis A virus (HAV) and an enveloped virus, herpes simplex 1 (HSV-1).

Methods: The antiviral potential of grape seed extract (GSE), blueberry extract (BE), essential oil of rosemary (EOR), thyme (EOT) and green tea extract (EOGT) cranberry extract (CE), was evaluated in solution during a viral pre-incubation on MNV-1, HAV and HSV-1 using plate assay method. Different concentrations were tested for the different compounds ranging from 100 to 200 000 ppm during a contact time of 90 min.

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Results: The tested concentration of GSE at 10 000 ppm was the most efficient in reducing MNV-1 infection *($p<0.05$)* by 2.85±0.44 log in solution compared to GSE at 100 ppm which reduced MNV-1 only by 0.58±0.25 log. For BE a reduction of 2.39has been observed at 15 000 ppm for MNV-1.
Significance: This project sheds light on potential new bioactive substances to control foodborne viruses.

P2-212 Transmission of Norovirus through Aerosolization of Vomiting

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Introduction: Norovirus is the major cause of foodborne disease in United States and aerosolization of norovirus via vomiting can result in rapid development of foodborne illness outbreaks.
Purpose: A scoping literature review was conducted to identify research gaps in the contribution of vomitus aerosolization to norovirus transmission.
Methods: Literature published between 2000–2022 was identified using PubMed, MEDLINE, Web of Science and Google Scholar using the following keywords: norovirus, Norwalk, Norwalk-like virus, vomit, vomiting, vomitus, foodborne illness and food safety. Studies were included if it involved aerosolization of norovirus through vomiting and was conducted in the U.S. All titles and abstracts were screened by a single reviewer using Covidence.
Results: A total of 440 unique articles were identified in the initial search. Of these, 422 were excluded based on a title and abstract review. The remaining 18 articles underwent a full review and 4 met the inclusion/exclusion criteria. Two of these articles focused on the mechanism of aerosolization of projectile vomiting in a controlled laboratory environment and found that aerosolized norovirus can travel to distances through projectile vomiting, contaminating food, water and contact surfaces thus accelerating outbreaks. One looked at the effectiveness of sanitation after vomiting in four retail establishments. Other looked at air contamination with norovirus in laboratory-based study from vomiting in closed settings (e.g., airplanes) and found that dispersing ability of norovirus depends on size of aerosols produced during vomiting suggesting a connection between vomiting and outbreaks.
Significance: There is limited data on transmission of norovirus through aerosolization of vomitus in norovirus transmission. More research needs to be conducted to improve our understanding of this transmission pathway, assess risk and develop effective control measures to prevent illness.

P2-213 Successful Removal by Spin Columns of Cytotoxic Residues from Chemical Neutralizers Used to Test the Efficacy of Disinfectants Against Infectious Human Norovirus

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Introduction: Human intestinal stem cell derived enteroid (HIE) cultivation system has been increasingly used to measure the infectivity of human norovirus. However, its application to evaluate the efficacy of disinfection products against norovirus is limited, primarily due to the higher level of cytotoxicity of these products on enteroid cells, which results in decreased assay sensitivity.
Purpose: To develop a method to neutralize the cytotoxicity of commercial disinfection products on HIE.
Methods: Three EPA List G disinfectant products (two hydrogen-based and one ethanol-based) were tested. Catalase or D/E broth were selected to be evaluated as chemical neutralizers. Three different spin columns (cellulose triacetate (CTA), polyethersulfone (PES) or hydrosart (HDS)) were tested to remove disinfectant residues. Specifically, 50 µl of each disinfectant was mixed with 940 µl of respective chemical neutralizer and 10 µl of infectious norovirus or DI water as a negative control. The chemically neutralized disinfectants were further purified using each of the spin columns following manufacturer's instructions. Monolayers of HIE were incubated in a 24 well plate with a chemically neutralized disinfectant or each of the spin column purified eluants for 1 h and 96 h at 37°C and 5% CO_2. Medium was then tested for the amount of lactate dehydrogenase (LDH) using a CyQyant cytotoxicity assay kit to determine cell mediated cytotoxicity.
Results: No infectious loss of replicating human norovirus was observed after processing samples on the 3 spin columns. Chemically neutralized disinfectants resulted in cytotoxicity of 12 to 20.8% of HIE monolayers. After cleaning by spin columns, cytotoxicity levels on HIE were reduced to 0.2 to 12.8% (CTA), 5.4 to 10.1% (PES) and 1.5 to 3.3 % (HDS).
Significance: Use of chemical neutralizers, followed by further purification by HDS column was the most effective in reducing cytotoxicity of disinfectants on HIE.

P2-214 A Metagenomic Approach to Shellfish Virus Testing

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Introduction: Bivalve shellfish naturally bioaccumulate waterborne viruses. Metagenomic techniques offer the potential to detect all virus pathogens within shellfish tissues as well as identify potentially anthropomorphic viruses indicative of human waste exposure.
Purpose: To develop a method to simultaneously identify all viruses potentially present in an oyster
Methods: Ongoing efforts have focused on extraction methods for viral RNA from oyster nucleic acid for metagenomics sequencing. Initial results from two sets of oysters from waters judged hygienic ("clean") and waters not acceptable for harvest ("dirty") were evaluated utilizing the GPTT method and illumine sequencing platforms. Investigation of potential extraction methods was also performed utilizing the GPTT and an ultracentrifuge method utilizing Tulane virus (TV) and murine norovirus as contamination test cases using the nanopore sequencing system.
Results: Initial results utilizing the GPTT method for viral RNA extraction followed by sequencing using the illumine machine gave a number of short but intriguing sequences suggesting the potential presence a number of exotic viruses in very low copy number particularly in the "dirty" oysters. Unfortunately they were too short (less than 100 bp) to be judged valid virus sequences. Also despite using the GPTT method designed to separate viral RNA from oyster sequences, the vast majority of sequences obtained were oyster derived (c.a. 475K of 500K reads). Subsequently the ultracentrifuge method followed commercial extraction and DNase digestion kits was tested. Results indicated that a substantial number of sequences were Tulane and MNV when this method was paired with nanopore sequencing which can generate contiguous sequences of 1000 bp of more.
Significance: Identification of both pathogenic and fecal indicator viruses may provide a better understanding of the anthropogenic impacts on shellfish beds as well as provide a means for identifying unknown viruses that may be contaminating shellfish.

P2-215 Enteric Virus Detection in Wastewater Influent and Effluent

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Introduction: Wastewater can indicate human specific enteric virus communal levels because it contains high viral excretions from those infected, and long environmental survival of these pathogens. Viral presence in wastewater effluent impacts receiving waters, thus shellfish growing areas and produce irrigation waters.
Purpose: This study determined the presence of norovirus genogroup I (GI) and genogroup II (GII) from influent, effluent wastewater during the typical increased enteric virus outbreak season in an Alabama wastewater treatment plant (WWTP).
Methods: Influent and effluent samples were grabbed weekly from an Alabama WWTP during January, February, and March. 800 ml of wastewater was analyzed for norovirus GI and GII using PEG precipitation and ultracentrifugation. Commercial extraction kits and optimized RT-qPCR assays were used extraction and detection, respectively. Twelve samples were analyzed in triplicate across the three months.

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Results: Norovirus GI was at the limit of detection in 25% of wastewater influent samples, with an average Ct of 43.32, and no detection in corresponding effluent samples. Norovirus GII was detected in 100%, average Ct of 41.32, and 67%, average Ct of 44.59, of wastewater influent and effluent samples, respectively. Estimated log reductions, based on relative Ct values, ranging 0.40- 2.20 were seen for norovirus GII in effluent. Minimal to no inhibition present across the RT-qPCR assays, as defined by less than three Cts from the negative internal control.

Significance: WWTPs reduce the risk of potentially infectious enteric pathogens, but conventional wastewater treatment alone does not eliminate contamination. Viral presence in effluent potentially impacts receiving waters used in shellfish growing areas and produce irrigation, thus negatively impacting food safety and public health. Reducing the microbial contamination, as this type of data shows, along with appropriate safeguards, like national sanitation guidelines, aids the determination of appropriate food safety control measures for food harvest and reduces or eliminates the contamination of foods.

P2-216 Viral Detection by qPCR and Digital PCR on Berries after Long-Term Storage

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Undergraduate Student Award Entrant

Introduction: Frozen berries have been implicated in outbreaks of foodborne illness attributed to viral contamination. Frozen berries have a long shelf life, which amplifies the need for data regarding virus detection on frozen berries over time.

Purpose: The objective of this multi-part study is to assess the persistence of Hepatitis A (HAV) and Tulane virus (TV) on frozen berries using quantitative polymerase chain reaction (qPCR) and digital PCR (dPCR) to better evaluate risk.

Methods: HAV and TV were inoculated at 10^5 viral particles on strawberries and raspberries (15g/sample) and frozen over 690d. Viruses were eluted by rinsing the berries with 10mL 1XPBS every 30d. Rinsates were previously assessed for viral infectivity and stored at -80°C for 3 years. RNA was extracted from previously collected samples using the Qiagen RNeasy Power Microbiome kit (n=48 rinsates per berry type). HAV and TV RNA were analyzed by qPCR and dPCR.

Results: HAV was quantified through qPCR at $7.8\text{x}10^5$ and 2.3×10^5 copies/mL (p<0.35) on raspberries, and 5.9×10^5 and 1.4×10^5 copies/mL (p<0.42) on strawberries, at 30 days and 690 days, respectively. TV was detected at 1.9×10^6 and 1.3×10^5 copies/mL (p<0.0001), and 3.6×10^5 and 5.2×10^4 copies/mL (p<0.98), on raspberries and strawberries at 30 days and 690 days, respectively. These qPCR values were associated with CT values within the range of 25-32. Through dPCR, HAV was detected on raspberries at 1.35×10^6 and 5.16×10^5 copies/mL ($P<0.0008$), and on strawberries at 1.3×10^6 and 2.2×10^5 copies/mL ($P<0.0029$), at 30 days 690 days, respectively. TV was detected on raspberries at 1.3×10^6 and 2.7×10^5 copies/mL ($P<0.0005$), and on strawberries at 4.9×10^5 and 8.1×10^4 copies/mL ($P<0.0002$) for 30 days and 690 days, respectively.

Significance: This project provides insight into RNA quantification by qPCR and dPCR along with HAV and TV RNA presence and infectivity on frozen berries.

P2-217 Improvement in the Detection of Murine Norovirus and Hepatitis A Virus from Post-Washing Water Containing Gallic Acid and Soil-Originated Various PCR Inhibitors

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Introduction: Plant- and soil-originated polymerase chain reaction (PCR) inhibitors like polyphenols, polysaccharides, and humic acid can impact the sensitivity of detection of low-concentration foodborne virus in post-washing water (PWW).

Purpose: The current study aimed to enhance foodborne virus detection in PWW containing various PCR inhibitors.

Methods: Two log PFU/ml of murine norovirus (MNV) and hepatitis A virus (HAV) were inoculated into water containing gallic acid (0.625, 0.125, 0.25%, *m/V*), and soil-rich (5% sandy loam, *w/V*) water containing starch (1%, *m/V*), pectin (0.00625%, *m/V*), humic acid (1 mg/L, *m/V*), and gallic acid (0.125%, *m/V*). Virus recovery was performed by using the paper filtration-coupled ultrafiltration (PFC-UF) combined with PCR inhibitors removal kits or polyvinylpyrrolidone (PVP) RNA extraction method and quantified by RT-qPCR.

Results: Compared with the Rneasy mini kit, the PVP method does not enhance the detection of MNV and HAV with the removal of gallic acid. Under the rich-soil (5%, NTU>800) and various inhibitors background, 17.58±7.28% of MNV-1 (9/9) and 7.37±4.06% of HAV (9/9) was recovered by using OneStep PCR Inhibitor Removal Kit.

Significance: This provides an effective way to enhance the low concentration of virus recovery in the complex background of PWW. However, risk assessment of low viral concentration from single rich PCR inhibitors background is still challenging.

P2-218 Next Generation Sequencing for Whole Genome Sequencing of Hepatitis A Virus Directly from Food Samples

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Introduction: Next-generation sequencing-based whole genome sequencing (WGS) was an influential approach to identifying viral pathogens in microbial research. However, the direct application of WGS to foodborne viruses was still challenging due to the many inhibitors from the food matrix and low virus titers compared to clinical samples.

Purpose: This study aimed to develop the WGS method for analyzing the HAV genome of clams using the Illumina Miseq system.

Methods: The obtained HAV genome and genetic characteristics based on the phylogeny were compared. Eight HAV complete genomes were obtained from twelve serums, and nine complete genomes were obtained from twenty-three manila clams.

Results: Eight HAV complete genomes were obtained from twelve serums, and nine complete genomes were obtained from twenty-three manila clams. As a result of analyzing, eight HAV-IA and one HAV-IB were genotyped in the manila clam. All serum samples were HAV-IA type. The HAV-IA strains identified in the manila clam shared 95.2% sequence identity. The sequence identity between the strains from clams and serum was 93.6~93.8% for HAV-IA and 94.6% for HAV-IB.

Significance: The method of viral WGS in food samples may contribute to rapid genotyping, virus evolution, and epidemiological surveillance in foodborne virus outbreaks.

P2-219 Examining the Effect of Organic Acids on Inactivation of Hepatitis E Virus

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Introduction: Hepatitis E virus (HEV) causes acute hepatitis with approximately 20 million cases per year globally. While HEV is endemic in some developing countries, it is considered an emerging foodborne pathogen in developed countries. The transmission of HEV-3 has been shown to be zoonotic and mainly associated with the consumption of raw or undercooked pork products. We have demonstrated that certain marketed pork products were positive

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for the genome of HEV-3. Organic acids, such as acetic acid and citric acid, can be used under specific conditions to reduce microbial contamination in meat products.

Purpose: In this study, we investigated the application of citric acid and acetic acid to inactivate HEV-3 on food and on food-contact surfaces.

Methods: Plastic, stainless steel and pork pâté surfaces were inoculated with HEV-3, dried for 30 minutes and were treated with acetic acid or citric acid in triplicates at 1%, 3%, or 5%. The latter concentration is the maximum allowed in food industry. Following 30 minutes incubation, the infectivity of viral particles was determined by cell culture, using A549/D3 human lung carcinoma cells.

Results: A greater than two-log reduction in viral infectivity was observed on plastic and stainless steel treated with the organic acids, but the treatment was much less effective on HEV infectivity on pork pâté (average reductions of 0.47 log citric acid, and 0.63 log acetic acid).

Significance: Our data demonstrate that 1 to 5% citric and acetic acids show some efficacy in the inactivation of HEV on food contact surfaces. However, their inactivation potential is drastically reduced in a meat product. Therefore, they cannot be used for HEV-3 risk mitigation in high-risk food commodities for HEV contamination. The obtained data will help with establishing proper measures for prevention and control of foodborne transmission of HEV.

P2-220 Preservation Methods for Long-Term Storage of Foodborne Viruses

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Introduction: Several physicochemical treatments such as cryopreservation, lyophilization, and preservatives are applied to preserve tissues, cells, and microorganisms. Preservatives combined with cryopreservation and lyophilization are generally used for long-term preservation. However, preservation methods for foodborne viruses have not yet been developed.

Purpose: The aim of this study was to develop a long-term virus preservation method by physicochemical treatments using the hepatitis A virus (HAV).

Methods: Freezer (-20°C), deep freezer (-80°C), and lyophilization were determined as the physical treatment methods. Preservatives were prepared as follows: proteins (4% bovine serum albumin and 10% fetal bovine serum), 5 and 10% saccharides (sorbitol, sucrose, and trehalose), water-soluble polymer (10% polyethylene glycol, 5% polyvinyl pyrrolidone), 10% dimethyl sulfoxide, 10% glycerol, and 0.05% sodium glutamate. HAV was stored for 7 months and titrated using a 50% tissue culture infective dose ($TCID_{50}$) assay. The final concentration of HAV treated with preservatives was 7.5 log $TCID_{50}$/mL.

Results: HAV treated with 10% sorbitol, 5% sorbitol and sucrose and trehalose, 10% FBS and 10% trehalose, 5% PVP and 10% sorbitol, and 5% PVP and 10% sucrose showed 0.2 to 0.7 log and 0.2 to 0.3 log reduction at -20 and -80°C for 7 months, respectively. However, HAV without preservatives was reduced above 1.3 to 2.3 and 0.5 to 1.0 log at -20 and -80°C , respectively. At the lyophilization process, HAV was decreased to 0.8 to 2.9 log for 7 months. HAV treated with 5% sorbitol, 10% sorbitol, 5% trehalose, 5% sorbitol and sucrose and trehalose, 10% FBS and 10% sucrose, 10% FBS and 10% trehalose, 5% PVP and 10% sorbitol, and 5% PVP and 10% trehalose showed 0.3 to 0.6 log reduction.

Significance: Long-term preservation of cultivated viruses and foods and environment-isolated viruses is important to study viral pathogenesis and the epidemiological characteristics of viral strains.

P2-221 Evaluation of a PCR Amplification Method Based on *Cyclospora cayetanensis* Mitochondrial Genome

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Introduction: A method for detection of *Cyclospora cayetanensis* which relies on conventional PCR followed by sequencing was recently developed. This method can detect approximately five to 10 oocysts of *C. cayetanensis* in produce and six to12 oocysts of *C. cayetanensis* in agricultural water samples.

Purpose: Evaluation of a method for the detection of *C. cayetanensis*, based on the mitochondrial genome, using a library of DNA from *C. cayetanensis*-positive samples containing different amounts of DNA.

Methods: A total of 24 samples were previously tested using qPCR according to the BAM chapter 19c. Libraries were separated in five groups according to the Ct values (22.58 to 36.35). Samples were amplified with the 3F1 and 3R1 primers and amplicons were visualized in *QIAxcel* Advanced system. Samples were purified and sequenced. DNA sequences were aligned with the database and analyzed to verify if all samples were able to confirm the presence of *C. cayetanensis* DNA.

Results: None of the PCR assays amplified DNA from the negative controls. All the samples from groups 1, 2, and 3 were able to be confirmed with this mitochondrial mit3 PCR. Groups 4 and 5 (Ct range of 34.66-36.35) presented partial confirmation. Results obtained in groups 4 and 5 can be explained because those samples are in the fractional level in which, according to the FDA MMVS guidelines, 25 to 75% of the samples need to present positive results.

Significance: Confirmatory methods can be used to support findings using the *C. cayetanensis* regulatory method; however, these methods should be based on different gene targets and/or different molecular detection technologies that provide further information. In this study, we were able to confirm positive samples obtained with the standard detection method (BAM Chapter 19c) with the recently published method based on the mitochondrial genome (mit3). These results support public health and the FDA mission to support findings requiring regulatory actions.

P2-222 Investigate the Effect of Plasma Activated Water (PAW) on the Safety and Yield of Romaine Lettuces (*Lactuca sativa* L. var. *longifolia*) from Hydroponics

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Introduction: Hydroponics is susceptible to cross-contamination due to the use of nutrient-enriched circulated irrigation water. Chemical sanitizers, such as hydrogen peroxide-based sanitizers, are typically used to inactive the bacteria in hydroponics, however, the sanitizers can also damage the roots of the crops, causing stunted growth or death. Plasma activated water (PAW) is an environmentally friendly sanitizer that has a good potential to be used in hydroponics due to its minimal damage to the roots of plants.

Purpose: Test the feasibility of using PAW as a sanitizer in hydroponic to improve the safety and yield of lettuces.

Methods: PAWs were produced by a customized submersible dielectric barrier discharge (DBD) plasma reactor using air or nitrogen as the carrier gas. Romaine lettuces were grown in ebb-and-flow hydroponic systems and PAW, 3% hydrogen peroxide water, or DI water was circulated in the system for 20 min once a day. The lettuces were harvested after 21 days and the yield by wet weight and total plate count were measured.

Results: The results have shown that the average wet weight of lettuces from the hydroponic system treated with PAW, hydrogen peroxide water, or DI water was 89.5 g ±11 g, 70.8 g ± 8 g, or 87.9 ± 13 g, respectively. The yield in the hydrogen peroxide-treated system was significantly lower than the one from the other systems ($p<0.05$). The population of the bacteria on lettuces from PAW or hydrogen peroxide-treated lettuces was significantly smaller than the ones from DI water-treated system, which are 2.1 ± 0.12 log CFU/leaf and 4.8 ± 0.9 log CFU/leaf.

Significance: The research indicated that PAW has the potential of reducing the bacterial load transferred to the produce in hydroponics while did not significantly influence the yield.

P2-223 Methodological Differences Confound One-Size Fits All Approaches to Agricultural Water Management

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Introduction: Despite the fact that methodological differences affect observed microbial water quality, multiple sampling and sample processing protocols are referenced in the water safety literature. Research is needed to determine how these differences impact the comparability of findings, and the ability to perform meta-analyses using data from multiple studies.

Purpose: This study aims to (i) quantify the impact of methodological differences on observed microbial water quality, (ii) determine if these methodological signals can be disentangled from regional, water type, and other signals of interest, and (iii) determine how specific methodological differences (e.g., in sample type, in sample filter type) are associated with microbial water quality.

Methods: A 2,429,990 sample dataset representing >100 studies was compiled; each sample had data on at least one microbial water quality target (e.g., *Salmonella* presence, fecal indicator bacteria [FIB] levels). Variance partitioning analysis (VPA) was used to determine the variance in each microbial water quality attributable to environmental versus methodological factors, while the strength of the association between individual environmental and methodological factors were quantified using condition forest (CF) and regression.

Results: VPA showed that environmental and methodological signals could not be disentangled for pathogenic *Escherichia coli*, *Salmonella,* and *Listeria*, limiting our current understanding of, and ability to base guidance on foodborne pathogen ecology in freshwater systems. For example, 18% of variance in *Salmonella* detection was jointly attributable to methodological and non-methodological factors. Despite this, opportunities for site or waterway-specific management exist because substantial variance in microbial water quality was still uniquely attributable to environmental factors (e.g., 13% for *Salmonella*). In contrast, FIB levels were more strongly associated with environmental, as opposed to methodological factors, by CF.

Significance: Our findings suggest that metadata collection and management should be standardized across studies, with a minimum set of attributes, and comparable data is needed to develop water quality risk assessments.

P2-224 Characterization of P(MTAC-AAm)/Chitosan Composite Hydrogels: Rheology, Texture, Antibacterial Activity and Cytotoxicity

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Introduction: Bacterial infection is a serious environmental problem posing great threats to the safety and healthcare of our daily life, as it is spreading over various aspects such as drinking water, medical devices, food packaging, and so on.

Purpose: The objective of this study was to synthesize and characterize P(MTAC-AAm)/Chitosan composite hydrogels with strong antibacterial activity against *E.coli*.

Methods: A mixture solution of chitosan, MTAC, and acrylamide were prepared, followed by the sequential addition of crosslinker BIS and initiator APS under N_2. The obtained solution was sealed and placed in a water bath at 65°C for 2 h to completely crosslink, followed by rising with deionized water. Fourier transform infrared spectroscopy, X-ray diffraction, thermogravimetric analysis and scanning electron microscope were used to characterize the physicochemical properties, and the rheological and mechanical properties were studied by a Rheometer and a Texture Analyzer. The inhibition zone was tested to determine the antibacterial activity and the cytotoxicity assay was investigated via MTT method against normal VERO and lung cell lines.

Results: Based on the comprehensive characterizations, the composite hydrogels were successfully synthesized and showed a good thermal stability. The shear thinning behavior suggested that the composite hydrogel had non-Newtonian pseudoplastic fluid characteristics. The storage modulus increased with increasing angular frequency and was greater than loss modulus, revealing that hydrogels exhibited a typical weak gel behavior. This hydrogel in a cylindrical form could withstand a high compression strain of 85% without breaking and immediately recovered its original shape after removing the compression force. Strong antibacterial activity against *E. coli* was also evidenced, and no cytotoxicity was observed at a concentration lower than 125 μg/mL.

Significance: The as-prepared P(MTAC-AAm)/Chitosan composite hydrogels exhibit promising features for applications in water treatment in the food industry.

P2-225 Validation of Peroxyacetic Acid and Chlorine as Treatments for Agricultural Surface Water Used for Produce Post-Harvest Uses

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Introduction: Surface water provides a more inexpensive and readily accessible source of water for agricultural use; however, exposure to the environment makes surface water prone to microbial contamination and, therefore, unfit for post-harvest activities.

Purpose: This study evaluated the effectiveness of peroxyacetic acid and chlorine at reducing *E. coli* in rain barrel and creek water sources to satisfy the Food Safety Modernization Act requirement of 'no detectable generic *E. coli'* in water used post-harvest on produce.

Methods: Water obtained from a creek and rain barrel were inoculated with a generic *E. coli* cocktail (~5 log CFU/ml) and equilibrated to 32 and 12°C to mimic varied seasons. Equilibrated samples were treated with ambient sterile distilled water control (W), peroxyacetic acid (PAA) 75±5 ppm, and chlorine (Cl) 25±2 ppm. Dey-Engley broth was used to neutralize samples before enumerating *E. coli* populations at 0, 5-, 10-, 60-, 1440-, and 2880-min post-treatment using *E. coli*/coliform (EC) Petrifilm and the Colilert method. Neutralized samples were enriched to evaluate presence/absence of generic *E. coli*.

Results: The time x treatment interaction (P<0.0001) was significant for the Colilert method. For PAA, at 0 min post-treatment, *E. coli* was not detected, whereas for Cl, *E. coli* was detected at 10 min post-treatment (0.5 log CFU/ml), but not detectable 60 min post-treatment. PAA was more effective than Cl (P<0.05) at the 0- and 5-min post-treatment time points. However, when using the Petrifilm method, *E. coli* was not detected in samples treated with PAA or Cl at any time point.

Significance: Both PAA and Cl reduced *E. coli* within 60-min post-treatment. While additional research is necessary, this study provides initial data that allows growers and Extension educators to explore the use of these treatments for surface water sources if they are the only available option for use post-harvest in produce.

P2-226 Investigation of Microbial Water Quality of Irrigation Water in South Korea and Application of Water Disinfection Technologies for Irrigation Water

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Introduction: Irrigation water is one of the important risk factors for vegetable contamination with foodborne pathogens. Therefore, to ensure food safety, it is necessary to microbial disinfection technology for irrigation water.

Purpose: Investigation of microbial water quality in irrigation water and application of microbial disinfection technology for irrigation water.

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Methods: A total of 2,910 irrigation water samples (1,214 stream water, 1,567 groundwater, 95 reservoir, and 34 other water source samples) were collected from 1,026 sites from 2018 to 2022. Quantitative analysis of sanitary indicator bacteria (total coliform, *Eshcherichia coli*) was performed by membrane filtration method. To confirm the applicability of microbial disinfection technology to irrigation water, the effect of UV treatment and chlorine treatment on foodborne pathogens (*E. coli, Salmonella* spp., *Listeria monocytogenes*) in irrigation water was confirmed. The reaction solution was pretreated using a polymer agglomerating agent to confirm the disinfection efficiency according to the change in organic material content(pre-treatment level), and the treatment level was set to four stages: untreated raw water (agricultural water), pretreatment 50%, pretreatment 90%, and bottled water based on TOC(Total organic carbon).

Results: The concentration of total coliforms and *E. coli* by 3.1±1.1 and 1.2±1.2 log CFU/100 mL in stream water, 0.7±1.2 and 0.1±0.3 log CFU/100 mL in groundwater, 3.0±1.2 and 0.6±0.9 log CFU/100 mL in reservoir. In UV treatment, the UV dose required to deactivate the microbial 3 log(99.9%) was 2-2.6 mW/cm^2*sec under bottled water and 2.6-3.6 mW/cm^2*sec under irrigation water(stream water). In chlorine treatment, 2-3 logs(99%-99.9%) of pathogens were inactivated within 3 seconds under bottled water, and only 0.3 logs or less were found in irrigation water(stream water).

Significance: The contamination of sanitary indicator bacteria in irrigation water by water source was confirmed, and UV treatment technology was estimated to be effective in controlling foodborne pathogens in irrigation water.

P2-227 Evaluating Low-Cost and Low-Maintenance Methods to Improve the Biological Quality of Irrigation Water in Small Agricultural Producer Farms in Central Chile

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Introduction: Ensuring high food safety standards is crucial in the market. Microbiological contamination of vegetables, especially through irrigation with contaminated water, poses a significant risk for small-scale agricultural producers relying on surface water sources.

Purpose: This study aimed to evaluate two low-cost, low-maintenance filtration methods' effectiveness in reducing pathogen loads in irrigation water on small-scale producer farms under laboratory conditions.

Methods: Two sand filter models were developed and tested to assess their ability to reduce fecal coliforms, E. coli, and Salmonella in irrigation water used for vegetable cultivation. Water samples from an irrigation canal were artificially contaminated with 10^5 fluorescently labeled Salmonella isolates. Samples were collected before and after passing through the filters on a weekly basis for five weeks. Model 1 consisted of a single container filled with three types of sand, while Model 2 comprised three separate containers, each with one type of sand. Pathogen detection was performed using UFC plate counts and PCR targeting the invA gene. Fecal coliform and E. coli counts were determined through membrane filtration.

Results: Model 1 demonstrated an average reduction of 0.78 log units in Salmonella counts after passing through the filter, while Model 2 achieved a reduction of 0.97 log units. Both models exhibited average reductions of 41% (Model 1) and 33% (Model 2) in E. coli counts, as well as average reductions of 56% (Model 1) and 62% (Model 2) in fecal coliform counts. In contrast, the control channel, without any filtration system, showed no reduction in pathogen counts during the sampling period.

Significance: The results indicate that the tested filter systems effectively reduce the concentration of Salmonella, E. coli, and coliforms when irrigation water passes through them. Therefore, these filtration methods should be considered for implementation on producer farms to enhance the safety of the water used in vegetable cultivation.

P2-228 Development of a Rapid, Field-Based Assay for Detection of *Escherichia coli* O157:H7 in Irrigation Water

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Introduction: Contaminated irrigation water has been implicated in outbreaks of *Escherichia coli* O157:H7 in which leafy greens were the identified vehicle.

Purpose: A rapid field-based assay was developed for the detection of *Escherichia coli* O157:H7 in water used to irrigate fresh produce.

Methods: Five liters of tap water was dechlorinated and inoculated with *E. coli* O157:H7 at final concentrations of 10^2, 10^1, 10^0, and 10^{-1} CFU/ml. Duplicate plate counts were conducted on all inoculated water samples. The water was pumped (0.50 L/min) through Disposable K-Cup paper filters that were housed in a 3D-printed cartridge. Following filtration, the filters were removed from the cartridge, placed in a 250 ml Flask and enriched in Tryptic Soy Broth (TSB) at 37°C with shaking for 8 and 24 hours. Next, a swab was dipped into the enrichments, which were analyzed for the presence of *E. coli* O157:H7 using the PhageDx *E. coli* O157:H7 Easy Phage assay, which detects bacterial cells based on infection with luciferase reporter bacteriophages. Following completion of the assay, relative light units (RLUs) were read in a Hygiena EnSURE Touch hand-held ATP luminometer. Each sample was tested in duplicate.

Results: All samples tested positive for *E. coli* O157:H7 after both 8 and 24 hours of enrichment. After 8 hours of enrichment, RLUs ranged from an average of 1,619 for the original 10^{-1} CFU/ml sample to 20,000 RLUs for the 10^2 CFU/ml sample. After 24 h of enrichment, all samples produced RLU readings of 20,000.

Significance: Current generic *E. coli* irrigation testing requirements have not proved effective at indicating the presence of foodborne pathogens in water that was subsequently implicated in leafy green foodborne outbreaks. This research could lead to a field-based assay for direct detection of *E. coli* O157:H7 in irrigation water within 10 hours.

P2-229 Prevalence of *Salmonella* and Shiga Toxin-Producing *Escherichia coli* in Agricultural Water

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Introduction: Fresh produce is often associated to foodborne outbreaks as these products easily become contaminated with foodborne pathogens. At farm level there are several routes for produce contamination, including water.

Purpose: The aim of this research was to investigate the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* in surface water sources from produce farms in Georgia.

Methods: Monthly water samples (500 mL) from eight irrigation ponds were collected from February to December 2021 (N= 88). 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, *Salmonella* was detected in 6/88 (6.81%) water samples, from which 4 serotypes were identified: Saintpaul 3/6 (50%), Montevideo 1/6 (16.66), Mississippi 1/6 (16.66), and Bareilly 1/6(16.66). The STEC genes were detected in 86/88 (98.7%) samples, with prevalence varying amongst genes (*hly* 77/88 (87.5%), *fliC* 54/88 (61.63%), *eaeA* 28/88 (31.81%), *rfbE* 31/88 (35.22 %), *stx*-I 75/88 (85.22%), and *stx*-II 41/88 (46.59%)). The virulence genes for

STEC *stx*-I and *stx*-II were observed in 34/88 (38.63%) samples. *Salmonella* isolates were found in the summer months (May-Aug.), while STEC isolates were found through the whole sampling period.

Significance: The information from this research will represent a better understanding from the risk that agricultural water sources represent as potential source of produce contamination in the farm.

P2-230 Evaluating Alternative Water Reuse in Agriculture Using a Scientometrics Approach: 1992-2022

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Introduction: Climate change is an issue that affects the future of the agriculture industry. With seasonal variations exacerbated by climate change, factors such as drought have profound effects on agriculture. Globally, researchers are working on alternative technologies, including non-traditional water sources and their potential uses in irrigation.

Purpose: The purpose of this scientometrics study is to provide an analysis of research on using non-traditional water sources in agriculture worldwide to better equip researchers to see trends, identify research fields, and understand gaps in the research.

Methods: A comprehensive scientometrics study was carried out using data from 1992-2022. A search of scientific literature was done using Web of Science with keywords, such as 'irrigation water', 'produce-wash-water in agriculture', 'reclaimed water', 'irrigation-return-flow reuse', 'harvested rainwater'. The data set, comprising 18,986 publications was downloaded and cleaned to remove duplicates and redundancies. After cleaning, about 8000 publications were analyzed. The publications were sorted based on number of times cited, region of study, water source, and year of publication. The data was analyzed to indicate the variations and correlations between sources of water and the research groups.

Results: Our data analysis revealed that publications focused on reused water such as irrigation return flow, reclaimed water, and produce wash water. Alternative water sources are being researched in the US, China, Israel, and the Middle East. These publications have 15 to 213 citations, indicating a high interest in the research field. The publications were filtered based on author affiliations, indicating an interdisciplinary approach being used in combating climate change and water shortage.

Significance: The study provides a global mapping of alternate water reuse resources spanning over 30 years, which is significant for the research community interested in climate change, water quality, food safety, and policy implication areas in different countries. This information can help identify gaps that can be potentially filled by future research.

P2-231 Growers' Irrigation Practices, Knowledge, Trust and Attitudes Toward Wastewater Reuse in Lebanon, Jordan, and Tunisia through a Food Safety Lens

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Introduction: The uptake of treated wastewater (TWW) in agricultural systems is not yet to its full potential in the Middle East and North African (MENA) region.

Purpose: Alongside the legal and regulatory framework, the growers' compliance with safe practices and their awareness of water as a vehicle of pollutants to fresh produce needs to be assessed before promoting water reuse.

Methods: A convenient sample of growers (n=85) in Lebanon, Jordan, and Tunisia was recruited for a cross-sectional survey.

Results: The results showed that 70.6% of the growers did not participate in training on Good Agricultural Practices or similar programs and had limited knowledge about the transmission of pathogens (22.4%), COVID-19 virus (11.8%), pesticides (25.9%) and pharmaceuticals (24.7%) to food crops via TWW. Also, 65.9% believed TWW poses health risks to consumers when applied to crops eaten raw. Overall, a positive attitude toward TWW was a determining factor for maximizing water reuse applications. For 32.9%, limited accessibility to TWW and unavailability of treatment plants were among the primary reasons for not using it. More concerning, less than half controlled the quality and safety of irrigation water using microbiological tests (32.9%), chemical tests (37.6%), and turbidity tests (29.4%). Only 40% trusted local authorities' control of TWW quality, and 69.4% had no access to regulatory information.

Significance: These findings exposed the need to develop effective surveillance strategies and enhance the growers' awareness and capacities for controlling water quality to ensure safe water reuse and fresh produce safety.

P2-232 Environmental Factors Associated with *Salmonella enterica* Occurrence in Watersheds in Paraíba, Northeastern Brazil

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Introduction: Natural surface waters are potential reservoirs of *Salmonella enterica* that can contaminate foodstuff through irrigation.

Purpose: To investigate factors associated with the occurrence of *Salmonella* in surface water sources in Paraíba, northeastern Brazil.

***Methods:*:** A 5-month longitudinal study was performed in 15 sampling sites from the 3 largest watersheds in Paraíba. Water samples were collected in triplicate by modified Moore swab (MMS) and processed according to FDA-BAM. Temperature (TE), pH, conductivity (CO), salinity (SA), resistivity (RE), total dissolved solids (TDS), and dissolved oxygen (DO) were determined in situ. Other variables such as total rainfall in the respective municipality in the week before sampling (PLSMUN), on the day before sampling (PLDANM), on sampling day (PLDMUN), in the month before sampling (PLSMBC) and percentage of water storage in the respective weir on the sampling day (VOL) were also investigated. Binomial regression analysis and tree regression were performed with R.

Results: The overall frequency of *Salmonella* was 63%. A linear effect of TE was observed and identified as the most important factor affecting *Salmonella* occurrence, followed by PLSMUN, CO, and VOL. According to the regression tree analysis, *Salmonella* occurrence was higher (74% *vs.* 35%) in water below 33°C. In this condition, higher occurrence (83% vs. 50%) was observed in samples with higher CO (> 1,281 µS). Higher PLSMUN (>5.9mm) and smaller VOL (78%) were also associated with *Salmonella* occurrence. On the other hand, PLSMUN was identified as a key factor in warmer water samples (>33°C), in which increased *Salmonella* occurrence was detected (59% vs. 8%).

Significance: Factors such as water temperature and total rainfall the week before sampling seem to be associated with *Salmonella* occurrence in natural non-recycled water sources. Environmental factors must be considered to understand *Salmonella* contamination in water sources.

Funding: Cooperative Agreement to Support JIFSAN.

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P2-233 Diversity and Antimicrobial Resistance of *Salmonella enterica* Serovars from Surface Water Sources in Northeastern Brazil

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Introduction: Although *Salmonella enterica* has been frequently reported in non-recycled water sources, there is a paucity of information regarding serovar diversity and public health implications related to the presence of this organism in watersheds worldwide.

Purpose: To investigate the diversity of *Salmonella enterica* serovars and antimicrobial resistance genes (ARGs) in three major watersheds in Paraíba state, Brazil.

Methods: A total of 149 water samples were collected from 15 different sampling points in the three largest river basins in the state for 5 months in 2022. Samples were collected by the modified Moore swab method (MMS) and processed according to FDA-BAM. Whole genome sequencing of confirmed *Salmonella* isolates was performed using MiSeq or NextSeq platforms. Serovars and ARGs were determined *in silico* by SeqSero and ResFinder from the Center for Genomic Epidemiology, respectively.

Results: *Salmonella* was isolated from 94 (63%) samples. Of the 17 different serovars identified, *S.* Saintpaul (17%), *S.* Infantis (15%), *S.* Rubislaw (11%), *S.* Gaminara (9%), *S.* Sandiego (7%), and *S.* Urbana (7%) were the most frequently found.. A high frequency and diversity of ARGs conferring resistance to aminoglycosides were detected: *aac(6')-Iaa* (91%), *aac(3)-VIa* (3%), *aph(3')-Ia* (3%), *aadA1* (2%), and *ant(3")-Ia* (2%). ARGs conferring resistance to tetracyclines (*tet(A);* 13%), sulfonamides (*sul1;*3%), fosfomycin (*fosL1;* 3%), and to antiseptics (*qacE;*3%) were also detected. Importantly, the plasmid-encoded quinolone resistance gene *qnrB19* was found in 21% of the isolates.

Significance: The high frequency and diversity of *Salmonella* serovars detected in non-recycled water sources in the major watersheds in Paraíba State warrant further investigations on their role as potential reservoirs of *Salmonella enterica*. Factors associated with the high frequency of ARGs must also be investigated, especially the genes conferring resistance to aminoglycosides and the plasmid-encoded quinolone resistance gene qnrB19.

Funding: Cooperative Agreement to Support JIFSAN (Salmonella Water Project).

P2-234 Phylogenetic Analysis of *Salmonella Enterica* of Surface Waters from Paraíba State, Northeastern Brazil

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Introduction: The frequent detection of *Salmonella enterica* in non-recycled surface waters requires in-depth epidemiological investigations to understand contamination events.

Purpose: To assess the genetic relatedness of *Salmonella enterica* isolates (n=147) from different surface water sources in Paraíba State, Northeastern Brazil.

Methods: A total of 149 water samples were collected from 15 different sampling points in the three largest river basins in the state for 5 months in 2022. Samples were collected by the modified Moore Swab method (MMS) and processed according to FDA-BAM. Genomic sequences were acquired on Illumina platforms. Phylogenetic relatedness was calculated from SNP matrices using the CSI Phylogeny server (https://cge.food.dtu.dk/services/CSIPhylogeny). Sequences were aligned in MUSCLE and the phylogenetic distances were determined by the Neighbor-Joining method with complete deletion of gaps and 1,000 bootstrap replicates to provide a measure of statistical support for the inferred relationships. The resulting tree was visualized in MEGA11. *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2 (GenBank accession number NC_003197.2) was used as the reference genome and *Shigella flexneri* 1235-66 (GenBank accession number AKNF01000780-1) was used as outgroup.

Results: The phylogenetic trees were mainly comprised of monophyletic lineages of the same serovars well supported by the bootstrap values (>90% bootstrap support). Clonally related isolates could be identified for the majority of the 18 detected serovars across time and location, including *S*. Rubislaw, *S*. Newport, *S*. Infantis, *S*. Muenchen and *S*. Urbana, although high genetic diversity was observed among isolates of some serovars.

Significance: The results suggest the occurrence of clonal lineages of *Salmonella enterica* across different surface water sources. The presence of clonal lineages from the same sampling points in different sampling periods indicate persistence of *Salmonella* strains in aquatic environment or frequent reintroduction events from external contamination sources.

Funding: Cooperative Agreement to Support JIFSAN (Salmonella Water Project).

P2-235 Detection and Antimicrobial Susceptibility of *Listeria monocytogenes* and *Salmonella* spp. Obtained from Chilean Watersheds

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Introduction: *Salmonella* spp. and *Listeria monocytogenes* (*Lm*) can contaminate produce through irrigation water. The spread of antimicrobial resistance (AMR) in foodborne pathogens through irrigation water represents an emerging concern in food safety.

Purpose: To identify and characterize antimicrobial susceptibility of *Lm* and *Salmonella* in four Chilean watersheds.

Methods: Surface water samples (n=1,050) were collected monthly from 30 sites in Maipo (MAI; n=240), Mapocho (MAP; n=270), Claro (CLA; n=270), and Lontue (LON; n=270) watersheds from April 2019 to February 2020 through the Modified Moore Swab technique. Irrigation canals (18/30-MAP and 17/30-MAI; 9/30-CLA and 10/30-LON) and rivers (10/30-MAP and 10/30-MAI; 15/30-CLA and 12/30-LON) were among the most frequently sampled sites. *Salmonella* spp. and *Lm* were isolated following modified FDA/BAM protocols. *Lm* (n=56) and *Salmonella* (n=153) isolates were sequenced in Illumina platforms, and antimicrobial resistance (AMR) genes were detected with Staramr. AMR was determined for a subgroup of *Lm* (n= 37) and *Salmonella* (n=57) from CLA and LON following CLSI recommendations.

Results: *Lm* and *Salmonella* were found in all watersheds. *Lm* y *Salmonella* prevalence was 21.8%(59/270) and 30.0%(81/270) for MAP; 25.0%(60/240) and 25.8%(62/240) for MAI; 21,5%(57/270) and 25.6%(69/270) for CLA; and 15.2%(41/270) and 28,5%(77/270) for LON, respectively. Co-detection of *Lm* and *Salmonella* was 5.9%(16/270) in MAP; 7.5%(18/240) in MAI; 7.8%(21/270) in CLA; and 5.6% (15/270) LON and occurred mainly in spring-summer 81% MAP(13/16), 71% CLA (15/21), and 67% LON(10/15). Irrigation canals showed the highest co-detection rate (30-56%) compared with river sites (10-30%). *Lm* was only resistant to ampicillin (5,3%; 2/37). *Salmonella* isolates were mainly resistant to tetracycline (10,5%; 6/57), and five AMR profiles were detected. The bioinformatic analysis identified AMR profiles consistent with phenotypic AMR.

Significance: *Lm* and *Salmonella* in irrigation canals may constitute a public health problem. Our results evidence the need for surveillance programs for environments linked to food production.

P2-236 Comparing Machine Learning Approaches' Identification of Key Drivers Influencing Populations of Generic *Escherichia coli* in Surface Waters in Florida

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Introduction: Surface waters are a known pathway for pathogen contamination of fresh produce. However, there is large variation in the microbial quality of surface water sources because they are open to the environment. Specifically in Florida's surface waters, there is poor correlation between bacterial levels, including *Salmonella* and generic *Escherichia coli (E. coli)*, and environmental factors such as precipitation. As a result of the limitations of linear methods, in order to understand what is driving variability of bacterial levels there is a need to consider nonlinear effects.

Purpose: The purpose of this study is to identify which environmental drivers are controlling the variability of bacterial levels in Florida's surface water.

Methods: We analyze the relationship between generic *E. coli* (pathogen indicator organism, n=909) concentrations in various types of surface waters (canal, pond, lake, river, streams) in Florida and their respective physicochemical water characteristics (pH, redox potential, water temperature, conductivity, turbidity), climatological conditions (air temperature, precipitation, Evapotranspiration, radiation, daylength, vapor pressure deficit), and landscape features (elevation, land use, human development, proximity to waste water treatment facilities). Our approach was to compare predictions from various machine-learning frameworks (Random Forest, Conditional Random Forest, XGBoost Regressor, XGBoost Regressor integrated with global sensitivity analysis) that consider nonlinear effects to identify key drivers influencing generic *E. coli* levels.

Results: There is consensus among the machine learning approaches that elevation, pH, conductivity, and turbidity are important drivers controlling the variability of generic *E. coli*. The type of surface water was not ranked as a significant driver by the XGBoost algorithm.

Significance: Weather events may be driving the transport of bacterial sources via runoff and affecting conductivity, turbidity, and pH in surface waters. Future directions include integration of key environmental drivers into a mechanistic model to inform challenges of predicting generic *E. coli* levels in surface waters and agricultural settings.

P2-237 Classification Model to Predict *Salmonella* presence in Surface Waters Using Longitudinal Data Collected in Central Chile from 2019–2022

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❖ ***Developing Scientist Entrant***

Introduction: Surface water used for produce irrigation may be contaminated with *Salmonella* spp. and other foodborne pathogens and represent a substantial burden for producers and public health. Therefore, identifying *Salmonella's* presence and evaluating the best strategies for mitigation is critical. Based on previous data, statistical models can be used to predict *Salmonella* contamination.

Purpose: To build a model to predict *Salmonella* spp. presence in water in central Chile.

Methods: The dataset included the results of *Salmonella enterica* isolation from surface water samples taken between 2019 and 2022 from the Maipo and Mapocho rivers in Santiago, Chile. We collected 1,017 observations with 27 variables such as water temperature, pH, and garbage, among other environmental factors registered when collecting samples at each site. The dataset analysis included: i) Data description analysis: We classified the data based on variable type and plotted the distribution of the variables. ii) Data pre-processing: we identified possible missing data and outliers in the database and ran statistics analyses. iii) Classification models: A Matlab script was coded, and the Classification Learner App was trained with 1,017 observations.

Results: There were more sites where *Salmonella* was absent (720/1,017) than present (297/1,017), with higher detection in 2020 than in other years. Based on the accuracy, the best model was the Support Vector Machine Kernel supported into the ROC curve, with an accuracy of 70.9%, compared with Bagged Tree and Coarse Tree. The sensitivity for the model was 92.0%, and the specificity was 94.3%, which means that the model could predict 92% of the positive sites.

Significance: The use of trained models based on the environmental conditions of a specific place can be a powerful tool to predict *Salmonella* presence in specific sites. This model could represent a tool to predict water events and develop mitigations.

P2-238 Occurrence, Genetic Diversity, and Virulome of *Salmonella enterica* in Surface Waters of Two Food-Production Regions in the State of Rio De Janeiro, Brazil

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Introduction: Surface waters (SuWa) of agricultural and livestock-producing areas may be a route for contamination of *Salmonella* in food products, potentially posing a risk for consumers and the local population.

Purpose: To assess the occurrence, genetic diversity, and virulome of *Salmonella enterica* in SuWa from food-producing regions of the Rio de Janeiro State.

Methods: Distinct geographical areas of the Rio de Janeiro State produce vegetables, eggs, chicken (Region 1, R1), and dairy products (Region 2, R2) dedicated to the regional market. We analyzed 72 SuWa samples from 54 sites in R1 and 101 samples from 52 sites in R2. Samples (10 L) were filtered *in situ* using the modified Moore Swab technique. *Salmonella* isolation and identification followed modified FDA/BAM procedures. Representative isolates selected based on RAPD were sequenced at CFSAN/FDA on the Illumina MiSeq or NextSeq platforms. Assembled genomes were used for *in-silico* serovar prediction (SISTR), MLST (Enterobase, GrapeTree) and SNP phylogenetic analyses (Snippy, RAxML), and virulence genes detection (Abricate, VFDB).

Results: *Salmonella* was detected in 58% SuWa samples of R1 and 53% of R2. A total of 178 non-clonal isolates were identified (R1=79; R2=99). Among 24 serovars, Newport, Typhimurium, Panama, Infantis, IV 43:z4,z24:-, and Sandiego were the most prevalent in R1. R2 isolates were distributed into 26 serovars, with Typhimurium, Newport, Panama, Carrau, Braedenrup, and Saphra as the most prevalent. Virulome analysis showed differences among these serovars; *S.* Typhimurium has the largest virulence genes repertoire, and IV 43:z4,z24:- the lowest. Phylogenetic analyses evidenced that the collection is highly diverse, suggesting varied sources in both regions, but the virulence profile was consistent in close related MLST-STs.

Significance: The occurrence of *Salmonella* in water sources of food production regions of Rio de Janeiro is a common phenomenon. This highlights the need for education in safe food preparation practices for the population.

P2-239 Genomic Characterization and Antimicrobial-Resistance Genes in *Salmonella* spp. Isolated from Surface Water in Brazil, Chile, and Mexico

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Introduction: Surface water (SuWa) could spread foodborne pathogens like *Salmonella*, which has been increasingly linked to outbreaks caused by produce. Moreover, *Salmonell*a carries antimicrobial resistance genes increasing the risk to public health.

Purpose: Perform genetic characterization of *Salmonella* isolated from surface waters in Brazil, Chile, and Mexico.

Methods: Isolates (n=1541) obtained from SuWa from Mexico (n=686) and Chile (n=592) (2019-22), and Brazil (n=263) (2020-21), were sequenced on Illumina platforms at CFSAN/FDA, and data was uploaded to the pathogen detection platform (NCBI). Ridom Seqsphere+ was used to determine sequence type (ST), and serotypes were predicted using SISTR v1.111. Antimicrobial-resistant genes (ARG) were detected with AMRFinderPlus.

Results: The collection showed a high ST diversity (Simpson index D=0.975). A total of 156 STs were identified, with three new STs discovered. The most common STs were 32 (7.2%), 13 (5.2%), and 19 (5.2%). Genomes of *S. enterica* subsps. *salamae*, *diarizonae* and *houtenae* accounted for 5.1% (78/1541) of the collection. All three countries shared 18/104 serotypes detected. Among them, epidemiologically relevant serotypes Newport (15.5%; 162/1541), Typhimurium (7.85%; 120/1541), and Infantis (7.4%; 114/1541) were the most frequently identified. Conversely, most *S.* Enteritidis originated in Chile (43/45) and were not detected in Brazil. Antimicrobial resistance genes were detected in 35.8% of genomes, and 39 (2.5%) genomes carried over 10 ARG. Notably, one genome from Mexico carried 16 ARG. Almost 50% of genomes from Mexico carried at least one ARG (340/686), but only 16% of genomes from Brazil carried ARG. Interestingly, three genomes carried the colistin resistance gene *mcr-9;* one *S.* Stanley from Chile and two *S*. Agona from Mexico. Finally, 11% of genomes (175/1542) carried beta-lactamase genes.

Significance: Resistant and multi-resistant *Salmonella* are present in surface waters used for irrigation, which may pose a risk for consumers. Mitigation measures might be required to prevent human disease.

P2-240 Stability of *E. coli* Concentrations throughout Ponds in South Georgia

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❖ ***Developing Scientist Entrant***

Introduction: Understanding the distribution of fecal indicator bacteria in irrigation ponds can help accurately quantify and interpret microbial water quality.

Purpose: To characterize seasonal patterns of *Escherichia coli* levels in irrigation ponds using mean relative difference (MRD) and assess physiochemical factors which affect *E. coli* levels using random forest (RF) analysis.

Methods: Water samples (0.5L) were collected bimonthly at set locations in three different irrigation ponds in South Georgia. Pond 1 was located on a cow-calf operation, and Ponds 2 and 3 were located adjacent to each other on a row crop farm. Surface water samples were collected with a grab sampler, and samples at 1.0 or 0.5m depth were collected using a peristaltic pump. Physiochemical water parameters were collected *in situ* using a multiparameter sonde, and most probable number (MPN) *E. coli*/100mL determined using Colilert with Quanti-tray 2000 MPN trays. Statistical analyses (MRD, RF) were carried out in R.

Poster

Results: At Pond 1 (n=572), MRDs for spring, summer, fall, and winter were correlated (p<0.05) with the overall MRD. At Pond 2 (n=528), summer, fall, and winter MRDs were correlated (p<0.05) with overall MRD. At Pond 3, spring, summer, and fall MRDs were correlated (p<0.05) with overall MRD (τ=0.36, 0.21, and 0.36 respectively). RF analysis showed chlorophyll and phycocyanin levels, specific conductivity (SPC), total dissolved solids (TDS), turbidity, temperature, and nitrogen levels had significant (p<0.05) influence on *E. coli* levels at Pond 1 (n=276). SPC, TDS, and pH had significant influence (p<0.05) at Pond 2 (n=264). SPC, TDS, and temperature were significant (p<0.05) factors for Pond 3 (n=144). Depth of sample was not significant (p>0.05) at any pond.

Significance: The MRD analysis indicates that patterns of *E. coli* may develop in irrigation ponds and persist across seasons. SPC and TDS significantly influenced *E. coli* levels in all three ponds.

P2-241 Metagenomic Survey of Antimicrobial Resistance in Surface Waters of Maryland across Diverse Land Use Designations

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Introduction: Antimicrobial resistance (AMR) is recognized as one of the most critical threats to public health world-wide. Surface waters are demonstrated AMR reservoirs and key integrators across human, animal, and natural environments and thus provide a valuable matrix for monitoring efforts. Sampling by land-use categories defined by the United States Geological Survey (USGS) provide a methodology by which to examine AMR by anthropogenic impact.

Purpose: To describe AMR, associated microbiomes, and pathogens of importance across Maryland surface waters defined as low and high human impact using metagenomic and quasimetagenomic next-generation sequencing (qmNGS) data.

Methods: Land-use designations were used to select 30 sites across the State of Maryland representing high (n=15) and low (n=15) "human impact" zones. Dead-end ultrafiltration was used to collect 20 liters of water from all sites. Metagenomic and quasimetagenomic (enriched) data were used with annotation pipelines (AMRplusplus, AMRfinderplus, CARD, FDA Kmer) to describe AMR and microbiota across Maryland surface waters.

Results: Thirty-three 'critically important' antimicrobial resistance genes (ARGs) from the National Antimicrobial Resistance Monitoring System list were identified by qmNGS data in 86% (26/30) of sampling sites. These belonged to: Colistin (1), Macrolide (5), β-lactam (15), and Fluoroquinolone (12) classes. Metagenomic sequencing identified five critically important ARGs in 20% (6/30) of sampling sites. Dominant bacterial taxa from enriched samples included *Aeromonas* spp. and *Vibrio* spp. as well as *Polynucleobacter* and *Synechococcus* for metagenomic data. β-lactam determinants were prevalent in twice as many high impact sites compared to low.

Significance: This study provides a valuable baseline survey of AMR across waters intersecting high and low human impact classifications for Maryland. These data can be used for future monitoring and evaluation and ultimately for improved stewardship of antimicrobials.

P2-242 Genomic Surveillance Reveals That Persistent *Salmonella* spp. Contamination of Surface Waters from Central Mexico Arises from Multiple Sources and Reintroduction Events

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Introduction: Surface water (SuWa) is considered an important vehicle for *Salmonella enterica* contamination of produce and food animals.

Purpose: To assess *Salmonella enterica* prevalence, persistence, and population diversity in surface waters used in agriculture and aquaculture production in Central Mexico.

Methods: We conducted 14 sampling rounds in 49 watersheds from May 2019 to August 2022. Overall, 689 SuWa samples were collected across six states of Central Mexico. Samples (10 L each) were filtered *in situ* using the modified Moore Swab technique, while *S. enterica* isolation and confirmation were performed following modified FDA/BAM procedures. Isolates were sequenced at CFSAN/FDA on the Illumina MiSeq or NextSeq platforms. Prevalence data were analyzed through meta-regression analysis and chi-square tests. Assembled genomes were used for *in silico* serovar prediction using SISTR version 1.1.1, and phylogenetic analysis by maximum likelihood using RAxML 8.0.

Results: S. enterica was detected in 63.4% of the samples (437/689), and 694 isolates were obtained. *S. enterica* prevalence varied across regions (X^2=27.7, *P*<0.0001) being lowest in Hidalgo (37.8, 95CI 15.1 to 61.7%) and highest in the State of Mexico (78.6, 95CI 65.1 to 89.9%). It also varied across seasons (X^2=11.4, *P*=0.0098): 76.5% in the rainy season and 58.8% in the dry season. *S. enterica* was repeatedly isolated in most sampling sites (42/49): average 9.2, 95CI 6.9 to 11.5 months. *S. enterica* serovar diversity was high (61 overall). Predominant serovars included Newport (77), Senftenberg (53), Anatum (43), and Typhimurium (31), which were disseminated across the six Mexican states. SNP phylogeny showed there was a high genetic diversity, although clonal dissemination (100% bootstrap support) of predominant serovars across time and regions was also observed.

Significance: S. enterica is commonly found in SuWa used for food production in Central Mexico, where it persists for extended periods (>6 months). The SE population structure suggests that SuWa contamination arises from multiple sources and reintroduction events.

P2-243 Antimicrobial-Resistance Susceptibility on *Salmonella* spp. Isolated from the Maipo River in Chile

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✦ ***Undergraduate Student Award Entrant***

Introduction: *Salmonella* contamination in agricultural waters may contribute to the transmission of this important foodborne pathogen. Surveillance is of utmost importance to understand the risk. It is also necessary to investigate the characteristics of *Salmonella* isolated from water, such as antimicrobial resistance, to properly identify public health hazards.

Purpose: This study investigated the antimicrobial susceptibility of *Salmonella* isolates recovered from the Maipo watershed.

Poster

Methods: We analyzed 74 *Salmonella enterica* isolates collected from 23 sites, including 17 irrigation canals along the Maipo River in central Chile. Isolates were collected monthly from April 2019 to January 2020. We analyzed the antimicrobial susceptibility to amikacin (AMI), amoxicillin/clavulanic acid (AMC), ampicillin (AMP), cefoxitin (FOX), ceftriaxone (CRO), ciprofloxacin (CIP), chloramphenicol (CLO), streptomycin (STR), gentamicin (GEN), kanamycin (KAN), trimethoprim/sulfamethoxazole (SXT) and tetracycline (TET) with the Kirby-Baüer methodology, Mueller-Hinton agar was used, the bacterial inoculum was prepared to a turbidity standard of 0.5 McFarland and 6 antibiotic discs per plate were tested, following the CLSI guidelines: Clinical & Laboratory Standards Institute.

Results: A total of 30% (22/74) of the isolates presented a multidrug-resistant (MDR) phenotype, 20% (15/74) presented resistance to 1-2 of the antibiotics tested, and 50% (37/74) were pan-susceptible. Isolates were more frequently resistant to CLO 32% (24/74), AMP 31% (23/74), and to CRO and STR with 28% (21/74) each. Isolates with the broadest resistance pattern showed resistance to TET-AMP-CRO-KAN-SXT-STR-CLO. We observed that resistant *Salmonella enterica* were obtained from 11/17 irrigation canals analyzed.

Significance: In this study, *Salmonella enterica* isolated from irrigation canals in the Maipo River in Chile presented resistance to multiple antibiotics, including important drugs used to treat complicated salmonellosis cases, such as third generation cephalosporins. The presence of *Salmonella* MDR, including resistance to clinically relevant antibiotics highlights the need to develop mitigation and control strategies for this pathogen in water sources.

P2-244 Zero-Valent Iron Reduces Non-Pathogenic *Escherichia coli* in Surface Water

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Introduction: Zero-valent iron (ZVI)-sand filtration can improve the microbial quality of irrigation water. However, little is known about the optimal ZVI exposure time needed to reduce foodborne pathogen levels.

Purpose: The objective of this study was to evaluate ZVI concentrations and exposure times needed to reduce levels of *Escherichia coli* TVS353.

Methods: Rifampicin-resistant *E. coli* TVS353 was inoculated (ca.6 log CFU/ml) into tubes with 9.8 ml of either autoclaved or non-autoclaved pond water and 10 g of a ZVI/sand mixture in concentrations of 0, 35, or 50% ZVI (v/v). Tubes were placed horizontally on a shaker and *E. coli* levels were determined after 1, 30, 60, or 120 minutes of exposure. Each treatment (water type, ZVI concentration, and exposure time) was performed in triplicate (n=72). *E. coli* were enumerated by spiral plating on MacConkey agar supplemented with rifampicin. Significant differences ($P<0.05$) in *E. coli* levels based on treatments were determined using Tukey's honest significance test.

Results: After 30 minutes of exposure, *E. coli* populations were significantly ($P<0.05$) reduced by 0.94 to 1.18 log CFU/ml at 35% ZVI; at 50%, populations were reduced by 1.03 to 1.30 log CFU/ml. *E. coli* populations after 30, 60, and 120 minutes of exposure were not significantly different at either 35% or 50% ZVI, except for autoclaved pond water with 50% ZVI at 30 minutes. After 120 minutes, *E. coli* populations in 35% and 50% ZVI were reduced by an average of 1.26 and 1.80 log CFU/ml, respectively. No significant reductions in *E. coli* were observed with 0% ZVI; similarly, no significant differences were observed between autoclaved and non-autoclaved pond water in all treatments.

Significance: Most of the *E. coli* reduction occurred within the first 30 min of exposure to ZVI/sand mixtures. These findings can be used to optimize filtration design to improve microbial water quality.

P2-245 Major Phytoplankton Functional Groups as Predictors of *E. coli* Concentrations in Agricultural Pond Waters

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Introduction: *E. coli* serves as a fecal indicator bacterium to assess the extent of fecal contamination. To date no study has evaluated if phytoplankton groups can serve as important predictors of *E. coli* concentrations in irrigation waters.

Purpose: Determine if phytoplankton functional group concentrations can be used as a predictor of *E. coli* concentrations in irrigation ponds using the random forest (RF) algorithm.

Methods: *E. coli* concentrations, phytoplankton functional groups (PFG), and water quality variables (WQV) were measured in two working irrigation ponds in Maryland, USA during the growing seasons in 2017 and 2018. A random forest (RF) algorithm was applied to the datasets in order to 1) assess the predictive capability of this algorithm and 2) evaluate the most important predictors of *E. coli* concentrations in the ponds.

Results: For both ponds, the WQV predictor set alone provided the best model performance metric results (R^2= 0.671 and 0.812, and RMSE= 0.321 and 0.374 log concentrations). The combined PFG and WQV predictor sets provided very close results to the WQV results alone and all in all the PFG variables alone as predictors showed the worst performance.

The top predictors in the PFG+WQV for Pond 1 were chlorophyll, total nitrogen, pH, turbidity, and dissolved organic matter which was similar to the WQV only set. Flagellates ranked among the most important predictors in the PFG+WQV (6th) and PFG predictor sets (1st). In Pond 2, the top predictors in the PFG+WQV were total carbon, temperature, pH, dissolved oxygen, and total nitrogen. Diatoms were found to be the leading predictor in the PFG-only dataset.

Significance: Results of this work indicate that sensed water quality variables serve as the most important predictors of *E. coli* concentrations in irrigation ponds but information on phytoplankton groups can marginally improve model performance.

P2-246 Spatial and Temporal Patterns of Microcystin Concentrations in Agricultural Pond Water

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Introduction: Cyanotoxins in agricultural irrigation waters pose a potential human and animal health risk. Cyanotoxins can be transported to crops and soil during irrigation where they can remain in the soils for extended periods and be absorbed by root systems. While many studies have reported spatiotemporal distributions for cyanotoxins in various freshwater sources, little has been reported for agricultural irrigation ponds.

Purpose: The goal of this study was to determine if persistent spatiotemporal patterns of microcystin occur in agricultural irrigation ponds.

Methods: The study was performed at a working irrigation pond during the summer of 2022 over six sampling dates. Concentrations of microcystin were determined using ELISA microcystin-ADDA kits. Ten water quality parameters were obtained using fluorometry and in-situ sensing. Relative differences (RDs) between a sampling location's microcystin concentration and average concentrations across the pond were computed and then averaged for each sampling location (MRDs). Spearman rank correlations were performed for both relative differences and actual measured values for water quality parameters and microcystin.

Results: Persistent spatial patterns of consistently higher and lower concentrations of microcystin were established. The pond's flow conditions and bank proximity to sample locations were indicative of the MRD values. The highest correlation coefficients were found between microcystin and pH (-0.777), and microcystin and phycocyanin (0.669). The lowest correlation coefficients were found for colored dissolved organic matter (0.226) and chlorophyll-*a* (0.289). Correlations between microcystin relative differences and water quality relative differences were generally low and not statistically significant.

Significance: Results of this work show that microcystin concentrations can exhibit stable spatiotemporal patterns in irrigation ponds, indicating that water quality sampling for cyanotoxins and placement of water intake should not be arbitrary. Research of the spatiotemporal organization of other cyanotoxin concentrations as well as understanding the degree of site-specificity of cyanotoxin concentration relationships with water quality parameters presents an interesting research avenue.

Poster

P2-247 Microbiological Quality of Bottled Mineral Water Commercialized in Bahia, Brazil

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Introduction: The negatives that many consumers have about tap water, along with the affordability and convenience of bottled mineral water (BMW) may be the main factors for obtaining these products as a primary source of water, making quality control imperative.

Purpose: The aim of this study was to evaluate the microbiological quality of 2180 BNW samples from different mesoregions of the State of Bahia, Brazil.

Methods: The samples were obtained in six years (2014 to 2019) and submitted to analyzes to determine the Most Probable Number (MPN) of *Pseudomonas aeruginosa* (PSA), *Clostridium perfringens* and *Enterococus* spp. by multiple tube method. In addition, total and thermotolerant coliforms were enumerated by membrane filter procedure. All data were analyzed using the Chi-square test (χ2). The significance level adopted was $p<0.05$, for a 95% confidence interval, using the statistical program SPSS® 21.0 for Windows.

Results: According to local legislation, 27.5% (599/2180) of the samples analyzed were found to be unfit for human consumption. Among the contaminations, 42.9% (257/599) occurred exclusively by total coliforms, in concentrations that varied from 1.0 to >2.0x 10^2 CFU/100 mL; Secondly, PSA was present in 22.8% (137/599) of contaminated samples; Then, *Clostridium perfringens* and *Enterococcus* spp. were detected in 3% (18/599) and 0.6% (04/599), respectively, and whose concentration ranged between 1.1 to >2.3 x 10 MPN/100 mL of water. The chi-square statistical test compared the failure frequencies and their incidence over the years, obtaining a statistically significant difference between them with $p<0.001$ where the year 2014 presents the highest percentage of failure (53.2%), while 2019 lowest rate (6.2%).

Significance: The data indicate the predisposition of BMW to microbiological contamination. This contamination is believed to be linked to the bacterial community present in the soil where the water well is located and/or to be introduced during industrial water processing.

P2-248 Occurrence of Indicator Genes of Antimicrobial-Resistance Contamination in the North Sea and English Channel Seawaters

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Introduction: The marine environment is a potential natural reservoir of antimicrobial resistance genes, subject to anthropogenic effluents (wastewaters, industrial, domestic) and known as a final receiving system. The *tetA, bla_{TEM}, sul1* and *intI1* genes have been proposed as indicators of contamination to assess the state of antimicrobial resistance in environment. Yet there is no information on their prevalence and abundance in large marine environments, far from the coast, such as the English Channel and the North Sea.

Purpose: To investigate the abundance and geographical distribution of the *tetA, bla_{TEM}, sul1* and *intI1* antimicrobial resistance indicator genes in the English Channel and the North Sea seawaters.

Methods: Bacterial DNA was extracted from 36 seawater samples collected during the IBTS oceanographic campaign in the English Channel and the North Sea. The absolute abundances of the indicator genes and the bacterial *tuf* gene (to evaluate the abundance of the bacterial population) were determined by qPCR and were analyzed in association with environmental variables and geographical locations to determine potential correlations.

Results: The *bla_{TEM}* and *tetA* genes were quantified in 0% and 2.8% of samples, respectively. The *sul1* and *intI1* genes were detected in 42% and 31% of samples, respectively, with an apparent co-occurrence in 19% of samples confirmed by correlation analysis. The abundance of these genes was correlated with the microbial population and environmental variables such as dissolved oxygen and turbidity. The highest abundances of the three *tetA*, *sul1* and *intI1* genes concerned the same sample that was collected from the West Netherlands coast area.

Significance: For the first time, we have shown the impact of anthropogenic inputs (rivers, man-made offshore structures, maritime activities) and environmental variables on the occurrence of indicators of environmental contamination by antimicrobial resistance in the North Sea and the English Channel seawaters.

P2-249 Isolation and Phenotypic and Genomic Characterization of Coliphages for Potential Use as a Water Quality Indicator

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Introduction: There is increasing interest in the use of coliphages (phages that infect *Escherichia coli*) as indicators of fecal contamination in ambient water.

Purpose: The isolation and characterization of coliphages from environmental sources, and assessment as potential water quality indicators.

Methods: Freshwater samples (1 L) were collected from Lake Wilcox in Richmond Hill, Ontario, Canada. Ninety milliliter aliquots were mixed with 10 mls of 10X tryptic soy broth containing *E. coli* K12 as a host, with shaking for 24 h at 37° C. Phage plaques were isolated from purified lysates using the double layer agar technique. Following phage DNA isolation (Norgen Biotek), libraries were prepared using the Illumina DNA prep Tagmentation and IDT for Illumina DNA/RNA UD Indexes. Paired end (2 x 150 bp) sequencing was performed on the Illumina MiniSeq system. Sequence reads were filtered (FastQC v0.11.9) and trimmed (Trimmomatic v0.39), and were assembled de novo using Skesa v2.4.0. Phage genomes were annotated using Prokka v.1.14.6. The phages were analyzed by transmission electron microscopy (TEM) and receptor mapping to determine host specificity.

Results: Initial analysis indicated that both coliphages were the same morphologically and genomically. TEM analysis indicated that the coliphages were T5-like. Receptor analysis demonstrated that the coliphages used the outer membrane protein tsx, commonly found on *E. coli*, as a receptor. Both coliphages had a genome size of 110348 bp. Additional genomic analysis indicated that the coliphages had 100% homology to phage Slur09, which was isolated from cattle manure in 2015 in the United Kingdom, indicating a wide geographic distribution of this phage type, and its association with animal feces.

Significance: Irrigation water sources impacted by fecal contamination have been implicated in produce-borne outbreaks of bacterial disease. This study highlights the potential use of coliphages as an indicator of fecal contamination in water used in food production.

P2-250 Recovery of *Arcobacter* Species from Agricultural Irrigation Water and an *in Vitro* Assessment of Their Effect on the Paracellular Permeability of Intestinal Epithelial Cells

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Introduction: *Arcobacter* spp. are recognized as emerging foodborne pathogens. Human infections associated with *A. butzleri, A. cryaerophilus,* and *A. skirrowii*, are likely from consumption of contaminated ready-to-eat vegetables, water, or undercooked meat. We isolated and sequenced *A. butzleri* and *A. cryaerophilus* isolates from different irrigation water sources used on farms in the Mid-Western US. Additionally, some isolates were tested for their potential to affect the barrier function (permeability), and viability of intestinal epithelial cells (Caco-2). Paracellular permeability changes, as indicated by a reduction in transepithelial electrical resistance (TEER), may reflect the potential of *Arcobacter* to disrupt the intestinal barrier and cause watery diarrhea.

Poster

Purpose: To characterize *Arcobacter* species isolated from irrigation water using Whole Genome Sequencing and assess their impact on enteric cell toxicity and permeability using Caco-2 intestinal epithelial paracellular permeability model.

Methods: Surface and well water samples were collected monthly from various farms in the Mid-West during the growing season (June-September 2019). *Arcobacter* species were isolated by a culture method and sequenced using the Illumina-MiSeq platform. Polarized Caco-2 cells were infected with *A. butzleri* or *A. cryaerophilus* at different MOI ranging from 10:1 to 100:1, and TEER was monitored using an epithelial voltohmmeter. Cytotoxicity was determined by the WST-1-based colorimetric assay for the quantification of cell proliferation and cell viability.

Results: Most of the *Arcobacter* isolated from surface water were *A. butzleri* while a few were *A. cryaerophilus*. *A. butzleri*, but not *A. cryaerophilus,* was found to be cytotoxic and caused a TEER reduction in polarized Caco-2 cells.

Significance: *A. butzleri* isolated from irrigation water may be cytotoxic and increase the barrier permeability in human intestinal epithelial cells. Further studies, including adherence and invasion tests, could help predict the pathogenic potential of these isolates, while transfer coefficient studies could evaluate their potential to transfer to RTE produce.

P2-251 Evaluation of the Treatment Efficacy at Drinking Water Production Utilities and Selected Distribution Networks, in Comparison to the Source Waters

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❖ ***Developing Scientist Entrant***

Introduction: Approximately four billion waterborne-associated cases and 3.4 million deaths have been recorded worldwide. Although water treatment strategies are mandatory to solve these issues, the recontamination possibility becomes another concern.

Purpose: The objective was to evaluate the treatment efficacy of Addis Ababa and Adama water treatment plants.

Methods: The following methods were used for comparison; *E. coli* (TBX method), ESBL *E. coli* (TBX (cefotaxime) method), *Salmonella* and *Campylobacter* conventional culture method, followed by RT-PCR confirmation. (NMKL, ISO). For the physicochemical parameters, Free Chlorine (only for outlets) using (Manual panel test), Turbidity (Manual turbidity meter), pH and Electric Conductivity (EC) (Manual Hanna meter) were applied per the Standard protocols.

Results: A total of 118 and 80 samples were collected for *E. coli, Campylobacter* and *Salmonella* species estimation in the selected sampling sites. *E. coli* was checked to be present in all source water samples but not in the treated samples (100% log reduction). The *Campylobacter* species revealed similar (100% log teduction) findings near the treatment plant compound. However, it became positive in some distribution networks 7/ 49 (14.3%). On the contrary, *Salmonella* species became negative before and after treatment. The tested ESBL *E. coli* species against the cefotaxime drug expressed resistance for few water sources 2/17 (11.7%). All tested physicochemical parameters revealed acceptable results after treatment, except turbidity, which showed a moderate increment in two treatment plants.

Significance: Based on our study, almost the two treatment plants have treated source waters effectively to satisfy their respective consumers' requirements. The continuous population growth, source water scarcity, growth of resistant genes in source waters and recontamination after treatment interventions were the most associated risks identified, which need attention. Water Quality and Quantity are the two main factors to safeguard the community from environmental contamination and outbreaks.

P3-01 Potential Hotspots of Antimicrobial Resistance Emergence and Dissemination in the Environment: A Case Study in Central Virginia

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✦ ***Undergraduate Student Award Entrant***

Introduction: Antimicrobial resistance (AMR) is a public health threat predicted to cause 10 million deaths annually by 2050. While the environmental component is speculated to contribute to the prevalence of AMR in bacteria, many dimensions of environmental antibiotic pollution and resistance are still unknown and require further research.

Purpose: The present study aimed to assess the variance of AMR prevalence in *Escherichia coli* isolated from different characteristics of environmental samples in Central Virginia.

Methods: Environmental samples, including feces of livestock and wild avian, water from wastewater treatment facilities, and water from drainage areas of different land use systems (crop, forest, pasture, and urban land), were obtained between August 2020 and February 2021. A total of 450 *E. coli* isolated were tested for their susceptibility to 12 antimicrobial agents approved by the US Food and Drug Administration for clinical use.

Results: Approximately 87.8% of the tested *E. coli* was resistant to at least one antimicrobial agent, with 3.1% of the isolates showing multi-drug resistance. Resistance to streptomycin was the most common at 73.1%. The most effective antimicrobial agent was chloramphenicol showing 97.6% susceptibility. One isolate obtained from a wastewater treatment facility was resistant to seven antimicrobials. The prevalence of AMR in *E. coli* isolated from the wastewater treatment facility was the highest, followed by those isolated from drainage area water, wild avian, and livestock. No significant (P>0.05) difference in AMR prevalence was found in *E. coli* isolated from different land use systems.

Significance: This study is the first documented research identifying potential hotspots of AMR emergence and dissemination. The findings will be helpful for policymakers and researchers to identify gaps in knowledge about the links between AMR and the environment. Continued research efforts on a larger scale are needed to confirm the environmental impact on the prevalence difference of AMR in bacteria.

P3-02 Bioactive Compounds and Biopreservative Potentials of the Essential Oils Obtained from *Eucalyptus camaldulensis* and *Azadirachta indica* against Foodborne Pathogens

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Introduction: Essential oils are natural extracts being explored as preservatives against food spoilage microorganisms and foodborne illnesses.

Purpose: Compounds from plant and herb extracts are being explored as natural antibacterial, antioxidants, preservatives, and therapeutic use.

Methods: Essential oils of *Azadirachta indica* (Neem) and *Eucalyptus camaldulensis* (Eucalyptus) were evaluated for antibacterial, antioxidant, and bio-preservatives potentials. Meat spoilage microorganisms were isolated and characterized phenotypically. The essential oils were extracted using hydro-distillation and the antimicrobial activity was assayed using agar well diffusion assay, the MIC and MBC were determined using broth dilution method. Bioactive compounds were evaluated using total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH), and ferric reduction antioxidant power (FRAP) methods. *Proteus penneri* was identified using 16SrRNA sequencing. Bio-preservative potentials were evaluated on meat spoilage by enumerating the total heterotrophic counts for 10 days at 4°C.

Results: The essential oils yield from 1000 grammes of Neem and Eucalyptus were 10.39% and 10.17% respectively. *Proteus* spp. were the most prevalent bacterial isolates obtained. The 100% Eucalyptus oil showed the highest antibacterial activity with zone of inhibition higher than 27.67±0.05mm and had the least MIC/MBC of 25µl/ml and 50µl/ml while 100% Neem oil had the weakest activity. The most susceptible bacterium was *Proteus penneri* N5 while *Staphylococcus aureus* S1 was resistant to all the essential oils concentrations. Neem essential oil (100%) had the strongest antioxidant capacity using TAC,

(Neem, 40: Eucalyptus, 60)% combination of the essential oils had the best DPPH activity while 100 % Eucalyptus oil had the highest FRAP. There was a 1 to 2 log CFU/g reduction in the meat challenged with *P. penneri* N5 and treated with Neem and Eucalyptus essential oils over a period of 10 days at 4°C. The essential oils possessed bioactive compounds, biopreservative and antioxidant potentials at dose-dependent concentrations.

Significance: Eucalyptus oil showed significant potential in preserving meat, which can be explored as biopreservatives, and antioxidants in foods.

P3-03 Phenotypic Expression of Cadmium Resistance in *Listeria monocytogenes* Isolated from Dairy Processing Facilities in British Columbia, Canada

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❖ ***Developing Scientist Entrant***

Introduction: Persistence of *L. monocytogenes* in food processing operations is a significant challenge facing the industry. The ability of certain strains to persist after cleaning and sanitation suggests a genetic competitive advantage. Cadmium resistance of *L. monocytogenes* has been suggested to correlate with tolerance to sanitizers.

Purpose: Characterize cadmium phenotypic response of persistent and transient *L. monocytogenes* isolates collected from five different dairy processing facilities over 10-year period.

Methods: Isolates (n=88) were grown in Mueller-Hinton broth (MHB; 5 log CFU/ml initial cell density) with and without cadmium salts ($CdSO_4$ or $CdCl_2$) in a 96 well plate. Optical density (OD_{595}) was measured (FilterMax F5) at 10-minute intervals during incubation at 37°C for 24 h. Minimum inhibitory concentrations (MICs) of *L. monocytogenes* Scott A (*cadA*+) and *L. monocytogenes* WRLP85 (*cadA*-) to cadmium salts were determined using two-fold dilutions (10.9-700 µM). Based on MIC results, all strains were screened in triplicate for phenotypic cadmium-resistance by comparing lag times when grown in MHB and MHB+$CdCl_2$ (43.8 µM). Whole genome sequence (WGS) analysis identified strains as persistent or transient and whether they carried *cadA* (>80% alignment).

Results: MICs of cadmium salts for Scott A and WRLP85 strains were 175 and 43.8 µM, respectively. Lag phase duration (LPD) of Scott A was significantly more delayed at 43.8 mM $CdSO_4$ (865±21 min) compared to $CdCl_2$ (760±6.8 min; *P* <0.05). All strains carrying *cadA* (n=67) were capable of growth in MHB+$CdCl_2$, but with a significant increase (149±55 min) in LPD (*P*<0.05, paired t-test). Strains without *cadA* (n=21) were unable to grow in MHB+$CdCl_2$. Persistent (n=63) and transient (n=4) *cadA*+ strains did not differ in LPD (*P*>0.05, two-sample t-test).

Significance: High-throughput phenotype screening of *L. monocytogenes cadA*+ and *cadA*- strains in the presence of 43.8 µM cadmium salts aligned with genomic expectations from WGS. Future studies will characterize strains by sanitizer tolerance.

P3-04 Phenotypic Resistance of *Escherichia coli* Isolated from Local and Imported Meats in Ghana

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Introduction: Resistance of *Escherichia coli* to antibiotics that are commonly used to treat same is a threat to public health. Albeit meats have been demonstrated to be sources of antibiotic resistant *Escherichia coli* and threatens human health.

Purpose: The study examined the phenotypic resistance of *Escherichia coli* isolated from local and imported meats in Ghana.

Methods: Escherichia coli was isolated according to US FDA, BAM (Feng et al., 2017) from 100 each of local and imported chicken, beef and pork. PCR amplification of the partial invasion A (*invA*) gene (~284bp) was used to confirm *Escherichia coli* (Kichana et al., 2022). Phenotypic antibiotic resistance test was done according to Bauer et al. (1996).

Results: Escherichia coli was isolated from local beef (67.0%), chicken (41.0%) and pork of (23.0%). Also, 66%, 53% and 45% of imported pork, beef and chicken, respectively were contaminated by *Escherichia coli*. *Escherichia coli* isolated from locally produced meats were highly resistant to amoxicillin (86.7%), tetracycline (73.3%) and trimethoprim (60%), but susceptible to imipenem (100.0%), ceftriaxone (70.0%) and gentamicin (70.0%). The multiple antibiotic resistance (MAR) index ranged from 0.1 to 0.7 and 22 different resistance profiles were observed. Resistant to 6, 5, 4 and 3 different antibiotics were 12.7%, 12.7%, 12.7% and 32.7%, respectively. For *Escherichia* isolated from imported meats, they were highly resistant to amoxicillin (71.7%), but susceptible to imipenem (98.3%), chloramphenicol (80.0%), gentamicin (78.3%), ciprofloxacin (76.7%), ceftriaxone (65.0%) and tetracycline (60.0%). The MAR index ranged from 0.1 to 0.6 and 17 different resistance profiles were observed. Resistant to 5, 4 and 3 different antibiotics were 8.16%, 6.12% and 10.2%, respectively.

Significance: The study creates the awareness that some locally produced and imported meats sold on the Ghanaian markets are contaminated by multidrug resistant *Escherichia coli*, therefore, adequate cooking of meats prior to consumption in Ghana.

P3-05 Withdrawn

P3-06 Presence of Antimicrobial Resistance Genes in *Escherichia coli* Isolates from Chicken Carcass Samples during the Slaughter

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Introduction: Antimicrobial resistance is a problem worldwide, affecting human and animal health and contributing mainly to therapeutic failure. Microorganisms such as *Escherichia coli* are excellent indicators of the actual situation of antimicrobial resistance, as they have ease of acquisition and transfer of resistance genes.

Purpose: This study aimed to investigate the presence of antimicrobial resistance genes in *E. coli* isolates from the chicken carcass during the slaughter.

Methods: We obtained 150 isolates of *E. coli* from chicken carcasses after bleeding (58 isolates), plucking (40), evisceration (32), and after chiller (20) in a large poultry slaughterhouse for export, with a capacity to slaughter over 500,000 birds per day, located in Brazil. We carried out the research of the antimicrobial resistance genes via polymerase chain reaction (PCR), with the evaluation of genes related to resistance to β-lactams - *blaTEM*; Gentamicin - *aac(3)-II*; Tetracycline - *tetA*; Ciprofloxacin - *qnrS*, Trimetoprim - *dhfrI*; Sulfonamides - *south1*; Chloramphenicol - *cmlA5* and *floR*. We employed descriptive statistics to characterize resistance data. For each sampling point, we calculated the percentages of isolates with resistance genes.

Results: The only genes that we did not detect in any of the isolates were the *qnrS* and *floR*. The genes *sul1*, *cmlA5*, and *dhfrI* were the most detected, with a percentage of 22%, 21.3%, and 12%, respectively. We found a low percentage for the *aac(3)-II* (2.6%), *tetA* (6.7%), and *blaTEM* (8%) genes. We detected more genes in the carcass after bleeding, where one isolate presented five resistance genes simultaneously (*blaTEM; tetA; dhfrI; sul1; cmlA5*).

Significance: The presence of *E. coli* resistance genes in a poultry industrial environment indicates its potential in the dissemination of antimicrobial-resistant bacteria. Acknowledgments: CAPES, CNPq.

Poster

P3-07 High Prevalence of Intermediate Resistance to Ciprofloxacin in *Salmonella enterica* Isolated from a Brazilian Poultry Production Chain

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Introduction: The emerging of antimicrobial resistance (AMR) in foodborne pathogens is a global concern, especially in *Salmonella enterica* from animal origin foods, such as poultry, demanding proper knowledge of AMR features.

Purpose: We characterized the distribution and diversity of antimicrobial-resistance *Salmonella enterica* that were isolated from a poultry production chain in Minas Gerais, Brazil, with special attention to ciprofloxacin and multidrug resistance (MDR).

Methods: S. enterica (n=96) of different serotypes and from different processing steps were subjected to broth dilution assay to estimate the minimum inhibitory concentration (MIC) for 12 antibiotics (8 classes) and screened using PCR for the presence of 17 antimicrobial-resistance genes. Ciprofloxacin resistance was further investigated through high-resolution melting qPCR (HRM-qPCR) and sequencing of quinolone resistance-determining region (QRDR: *gyrA*, *gyrB*, *parC* and *parE*).

Results: Isolates presented mainly resistance to ampicillin (11/96) and most presented intermediate resistance to ciprofloxacin (92/96). Roughly one-third (33/96) were resistant to streptomycin based on our interpretive criteria. Most strains resistant to streptomycin and ciprofloxacin were PCR-positive for *aphA* (51/96) and *qnrB* (94/96), respectively. Minor differences were identified in melting temperatures (T_m), and a Thr57Sr mutation was observed in *parC*. MDR isolates harboring *acrA* and capable of expressing the AcrAB-TolC multidrug efflux pump were resistant to ethidium bromide at 0.4 mg/mL.

Significance: The intermediate resistance to ciprofloxacin may be associated to *qnrB*, and the potential role of Thr57Ser mutation warrants further investigation. Acknowledgements: CNPq, CAPES (finance code 001), FAPEMIG and FUNARBE.

P3-08 Characterization of Soil and Lettuce Resistomes from Harvest through Storage in Modified Atmosphere Packaging

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Introduction: We have previously shown that soil microbial communities contribute to the lettuce phyllosphere microbiome. Leafy vegetables carrying bacteria that harbor antimicrobial resistance genes may provide a pathway for those genes to enter the human microbiome.

Purpose: Antimicrobial, metal, and biocide resistance genes (ARGs, MRGs, and BRGs) in soil and lettuce phyllosphere microbiomes were profiled and compared to determine possible transfer between soil and lettuce as well as persistence during lettuce storage.

Methods: Shotgun metagenomic sequencing was performed on a total of 225 samples consisting of surface soil, harvested lettuce heads, processed lettuce (cut and washed), and processed lettuce cold-stored in modified atmosphere packaging for five different harvests in Salinas, California. Sequencing was performed on an Illumina NextSeq platform generating paired-end 150 bp reads, and classification of resistance determinants in the sequence datasets was accomplished using MEGARes 2.0

Results: Overall, a higher number of resistance genes per million reads was found in processed lettuce after storage (9.8) compared to processed lettuce before storage (0.74) (Wilcoxon Rank Sum, $P<0.001$). MRGs were prevalent in both soil and lettuce, with copper resistance particularly high in lettuce. BRGs were most frequent in processed lettuce before storage. In the five separate harvests, between three and 59 different resistance gene alleles were identified in both soil and at least one lettuce sample. Omitting genes conferring both drug and biocide resistance, and including all samples, the greatest percentage of ARGs belonged to the beta-lactam class (30%) followed by aminoglycosides (6.7%), and specific allele sequences from both classes were observed in common between soil and lettuce.

Significance: These results provide insight into the transfer of antimicrobial resistance genes from soil to the lettuce phyllosphere and ready-to-eat packaged lettuce, thus their potential to enter the human food chain.

P3-09 Antimicrobial Resistance Assessment of *Staphylococcus Aureus* Isolated from Dairy Cattle

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Introduction: *Staphylococcus aureus* is associated with mastitis in cows. Animal-to-animal transmission occurs; therefore, preventive control measures are required. In addition, this organism produces toxins that may end up in milk for consumption creating a public health hazard.

Purpose: To test alternative concentrations of iodine to reduce *S. aureus* and to create an antimicrobial resistance (AMR) profile of *S. aureus* isolated from infected cows.

Methods: Mammary secretion samples were collected from six culled cows, isolating 30 *S. aureus* strains. Cow's udders obtained from an abattoir after their slaughter activities were appropriately prepared, sanitized, and inoculated with the *S. aureus* strains. The inoculated udder skin was treated with 0.25, 0.35, 0.5, 0.75, and 1% iodine solutions. Simulated dipping was tested for 15, 30, and 60 sec. In addition, *S. aureus* was enumerated by spread plating on TSA and incubating overnight at 37ºC. A one-way ANOVA was performed to compare treatments and a pairwise comparison T-test was adjusted by the Bonferroni method. For the AMR, the minimum inhibitory concentration was tested using Sensititre™ plates (GPN3F).

Results: Regardless of iodine concentration and dipping time, all treatments were effective ($P<0.05$) in reducing *S. aureus* from the udder skin. No difference was found between iodine concentrations and exposure time. However, after 60sec, a higher pathogen reduction was achieved. For the AMR profile, bacterial growth was observed in quinupristin/dalfopristin, daptomycin, linezolid, and Oxacillin+2%NaCl, but no isolates were categorized as resistant (CLSI guidelines).

Significance: Testing the effect of antimicrobial agents and sanitizers at different concentrations is essential to decide sanitizing procedures. Maintaining strict hygienic conditions during milking is required to prevent the spread of disease at the farm and avoid the spread of organisms affecting public health. Antimicrobial stewardship at the farm level is effective in preventing AMR.

P3-10 Antimicrobial Use Practices and Resistance of Zoonotic Bacteria in Goat and Sheep Farms

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Introduction: Antibiotic use in animal production is one of the major contributors of antibiotic resistance in our environment. Significant data gaps exist on antibiotic use practices in farming, particularly in goat and sheep production.

Purpose: The purpose of this study was to determine factors that are significant drivers for antimicrobial use and resistance in goat and sheep farming.

Methods: A structured questionnaire was carried out for goat and sheep producers and a multivariate probit model was used to assess respondents' knowledge and practices on antimicrobial use. Antibiotic sensitivity was performed using Kirby-Bauer disk diffusion test method.

Results: The response rate of the survey was 38.8% and 79.9% producers administered antibiotics to the animals while 20.1% did not. Age of producers (-0.016) and use of antibiotics on farm (-0.575) were highly significant ($p > 0.05$) in antimicrobial usage. Approximately 86.8 % administered antibiotics for treatment of sick animals, 6 % for disease prevention, and 7.2% for reduction of disease. Oxytetracycline (31.3%), penicillin G, (25.8%), and florfenicol (17.5%) were most frequently used antibiotics. For respondents that answered questions on record keeping, 51.4% kept up to date records while 48.6 %

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did not. About 73.5% of respondents consulted veterinarian on need basis while 11.9% never consulted. With a positive coefficient of 0.43, producers with prior BMP training were more aware of antibiotics overuse and development of antimicrobial resistance. *Escherichia coli*, *Shigella, Salmonella,* and *Staphylococcus aureus* displayed resistance to ampicillin (28.5%, 0%, 37.8%, 95%) and tetracycline (11.5%, 100%, 16% and 100%), respectively.

Significance: Our findings suggest that producers who do not administer antibiotics are aware of effects of antibiotic overuse and development of antimicrobial resistance. This study also suggests the need to educate farmers on management and biosafety measures considering high reported occurrences of diseased animals and usage of antibiotics in goat and sheep farms.

P3-11 Investigation of Antimicrobial Sensitivity in Bacteriophage-Insensitive Mutants of *Salmonella enterica*

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❖ ***Developing Scientist Entrant***

Introduction: Non-typhoidal *Salmonella* is a leading cause of foodborne diarrheal disease. The prevalence of antimicrobial resistant (AMR) isolates is rising, with overexpression of the AcrAB–TolC efflux pump often linked to multidrug resistance. We propose a targeted strategy to force the evolution of TolC by bacteriophage (phage) infection which we hypothesize will restore antimicrobial sensitivity.

Purpose: To assess changes in AMR of bacteriophage-insensitive mutants (BIMs) of *Salmonella* Typhimurium resistant to chloramphenicol, following targeted infection with a TolC-binding phage.

Methods: Using an in-house collection of newly-isolated phages (n=80), TolC dependence was determined via relative efficiency of plating using a Δ*tolC* mutant of *S.* Typhimurium. BIMs were generated by co-inoculating 5 µL of overnight culture with 5 µL of TolC binding phage (~10^9 PFU/ml, multiplicity of infection≈1) into 5 ml tryptic soy broth (TSB) in triplicate. After a 24-hour incubation (22°C; 180 RPM), 5 µl of co-culture and additional 5 µl of phage lysate (~10^9 PFU/ml) was transferred to fresh TSB. After three days, cultures were enumerated on streak plates and ten colonies were confirmed as phage resistant over five subcultures via spot test. Minimum inhibitory concentrations (MICs) of BIMs to chloramphenicol were determined via broth microdilution.

Results: One phage (phage #80) showed reliance on TolC as shown by an inability to form plaques on the Δ*tolC* mutant. Transmission electron microscopy revealed a rare Podovirus with C3 morphology. Host resistance to this phage arose after three days in broth culture. We observed enhanced sensitivity of BIM #5 (MIC=64 µg/ml) compared to the parent strain (MIC=128 µg/ml); however, this was the only reduction observed and is not sufficient to be deemed clinically susceptible.

Significance: This study demonstrates that forced evolution of the TolC receptor through phage infection is a potentially promising strategy for sensitization of AMR foodborne pathogens.

P3-12 Antimicrobial Susceptibility of Bacteria Isolated from Street Vended Foods in Maseru Lesotho

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❖ ***Developing Scientist Entrant***

Introduction: The nature and operational aspects of street food vending in Southern African countries create an ideal environment for food contamination, even with antimicrobial resistant pathogens.

Purpose: This study aimed to assess the antimicrobial susceptibility of selected pathogens isolated from street vended foods in Maseru, Lesotho.

Methods: The Kirby Bauer disc diffusion method using Muller Hinton agar was employed. Seven isolates; *E. coli, E. cloacae, E. asburiae, E. cancerogenous, C. freundii, S. paucimobilis* and *B. versicularies* were tested for antimicrobial susceptibility using amoxicillin (30µg), cefoxatime (30µg), cefpodoxime (10µg), nalidixic acid (30µg), chloramphenicol (30µg), tetracycline (30µg), gentamicin (10µg) and penicillin (10µg). The isolates were further tested for extended spectrum beta-lactamase (ESBL) production using the double disc synergy test. Cefoxatime (30µg), cefpodoxime (30µg) and amoxicillin/clavulanic acid (amoxicillin 20µg and clavulanic acid 10µg) were used for ESBL detection. *E. coli* (ATCC 13762) was used as a reference strain.

Results: All seven isolates were multidrug resistant (MDR) as they had resistance to at least two or more antibiotics. *E. coli* had the highest resistance (75%), followed by *C. freundii* (63%), whereas *S. paucimobilis* showed the lowest resistance (25%). Disc diffusion demonstrated that 57% of the isolates (*E. coli, E. cancerogenous, E. cloacae* and *C. freundii)* presented the ESBL phenotype. Additionally, extended spectrum β-lactamase activity was found in all the isolates, except for *S. paucimobilis*, *B. versicularies* and *E. asburiae*.

Significance: Antimicrobial resistance (AMR)is a worldwide public health issue. Humans may be infected with superbugs, therefore creating situations where treatment may be ineffective. These results highlight the need for action to prevent and fight AMR and potential risks faced by street food consumers in Maseru, where healthcare is already in a depreciated state.

P3-13 A Comparative Study on Antimicrobial Resistance in *Escherichia coli* Isolated from Channel Catfish and Siluriformes Products

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❖ ***Developing Scientist Entrant***

Introduction: Researchers are interested to learn whether aquaculture environments may become hotspots for antimicrobial resistance (AMR).

Purpose: To study AMR trends in *Escherichia coli (E. coli)* recovered from channel catfish and from Siluriformes data collected under the National Antimicrobial Resistance Monitoring System (NARMS) program.

Methods: This study analyzed AMR data on 114 *E. coli* isolates. Of these, 45 were from commercial-sized channel catfish harvested from fishponds in Alabama. Antimicrobial susceptibility testing (AST) and whole genome sequencing were performed using the GenomeTrakr protocol. Data for 69 isolates from Siluriformes products were also accessed from FSIS' NARMS program. To allow further investigations, sequence data for all 114 isolates were uploaded to the National Center for Biotechnology Information database under the bio-projects PRJNA357722 and PRJNA292667.

Results: The isolates from catfish from fishponds showed resistance to ampicillin (44%), meropenem (7%), and azithromycin (4%). The Siluriformes product isolates showed resistance to tetracycline (31.9%), chloramphenicol (20.3%), sulfisoxazole (17.4%), ampicillin (5.8%), trimethoprim-sulfamethoxazole (2.9%), nalidixic acid (2.9%), amoxicillin-clavulanic acid (1%), azithromycin (1%), and cefoxitin (1%). Eleven antimicrobial resistance genes (ARG) detected in the fishpond isolates showed no correlation with phenotypic resistance. However, the Siluriformes product isolates showed a statistical relationship between AST and ARG for folate pathway antagonists: sulfisoxazole vs. *sul1* and *sul2* (p=0.0042 and *P*<0.0001, respectively) and trimethoprim-sulfamethoxazole vs. *dfrA16* and *sul1* (*P*=0.0290 and *P*=0.013, respectively). Furthermore, correlations were found for tetracyclines: tetracycline vs. *tet(A)* and *tet(B)* (*P*<0.0001 each), macrolides: azithromycin vs. *mph(E)* and *msr(E)* (p=0.0145 each), phenicols: chloramphenicol vs. *mdtM* (*P*<0.0001), quinolones: nalidixic acid vs. *gyrA_S83L=POINT* (*P*=0.0004), and β-lactams: ampicillin vs. *blaTEM-1* (*P*<0.0001).

Significance: The different AMR profile of the two groups reveals more resistance to critically important antimicrobials (CIA) in humans and more ARG-AST concordance in *E. coli* isolates from Siluriformes products. To alleviate any public health concerns, further research on the occurrence of resistance to CIAs is warranted.

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P3-14 Evaluating the Effect of Broad-Spectrum Antibiotics in *Staphylococcus aureus* Biofilms Isolated from Bovine Mastitis

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Introduction: Biofilm-forming *Staphylococcus aureus* has been reported as a causative agent of bovine mastitis; it is a known toxin-producing foodborne pathogen causing disease if milk is consumed raw. Biofilm formation favors colonization causing chronic infections and enhances their ability to establish in dairy farms.

Purpose: Evaluate the effect of broad-spectrum antibiotics in *Staphylococcus aureus* biofilm formation and density.

Methods: S. aureus strains isolated from mastitic cows were used. Biofilm formation was tested using 96-well plates containing 100µl of Brain Hearth Infusion broth. Strains were inoculated in each well, treated with different antibiotics (Tobramycin, Levofloxacin, Imipenem, Meropenem, Doripenem, Amikacin, Gentamicin, and Ciprofloxacin), and incubated under static conditions for 48 hours at 37°C. Antibiotics were tested at various concentrations. Control wells contained *S. aureus* without antibiotics. Upon incubation, planktonic cells were rinsed with distilled water. The biofilm was stained with crystal violet, solubilized with ethanol, and transferred to a new plate to measure optical density (OD580nm) as an indication of biofilm density. The experiment was repeated three times.

Results: S. aureus biofilm density was 0.529±0.02. A significant difference ($P<0.05$) in biofilm density was observed between control and treatments. All antibiotics exhibited biofilm reduction; amikacin at 1µg/ml, 46.31%, and at 4µg/ml, 35.54%. Tobramycin reduced 58.79% at 0.12µg/ml and 72.02% at 1.0µg/ml. At 0.12 µg/ml and 1µg/ml of gentamicin, reduced 57.28% and 51.60%, respectively. With 0.016µg/ml and 0.06µg/ml of imipenem reduced 48.96% and 54.44%. Meropenem, at 0.03µg/ml and 0.12µg/ml, in 57.84% and 56.52%. Doripenem, at 0.016µg/ml and 0.06µg/ml, reduced 31.98% and 23.29%. At 0.06µg/ml and 0.5µg/ml of levofloxacin 59.17% and 23.44% of reduction was observed. Ciprofloxacin at 0.12µg/ml, 0.5µg/ml, and 2µg/ml; 51.42%, 31.57%, and 25.52% of biofilm reduction.

Significance: These experiments are part of larger research to identify treatments to control *S. aureus* in animals, humans, and processing environments. Understanding the effects of antibiotics in biofilm formation helps with treatment options to prevent and treat biofilm-associated infections caused by *S. aureus*, affecting public health.

P3-15 Virulotyping and Antimicrobial Resistance of *Salmonella enterica* Strains Circulating in Mexico

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✦ ***Undergraduate Student Award Entrant***

Introduction: The virulence of *Salmonella enterica* could be associated with their genetic and phenotypic characteristics and could be influenced by the origin of the strains.

Purpose: To evaluate the presence of virulence genes and the antimicrobial resistance of *S. enterica* strains isolated from different origins in Mexico.

Methods: A total of 115 *S. enterica* strains recovered from different sources [humans (n=12), environment (n=34), plant-based foods (n=37), panela cheese (n=13) and unspecified foods (n=19)] in Mexico were characterized. The antimicrobial resistance to 14 antibiotics was tested (amikacin, ampicillin, carbenicillin, cephalothin, cefoxitin, ciprofloxacin, chloramphenicol, gentamicin, netilmicin, nitrofurantoin, norfloxacin, sulfamethoxazole, streptomycin and tetracycline) by the Kirby-Bauer method. Also, the presence of 13 virulence genes (*agfA, orgA, spiA, sifA, sseF, sseL, invA, ssaQ, sspH1, sopE, pefA, spvC, hilA*) was evaluated by multiplex PCR. The strains were classified according to their resistance profiles and virulotypes.

Results: All strains showed the presence of nine virulence chromosomal genes tested, excepting an environmental isolate, which showed absence of *sseF.* From the mobile genetic elements, the most common was *sspH1* (91%; 105/115). Seven virulotypes were found. Virulotype V2 containing all nine chromosomal genes and *sspH*1 was the most frequent (85%; 98/115). V5 which contains all the genes tested, was present in 4.3% (3/115) of the strains, two from human origin and one from an unspecified food. According to the antimicrobial resistance, ampicillin was the most common (56%; 64/115). The 24% (27/115) of *S. enterica* strains were multidrug resistant.

Significance: Antimicrobial resistance and the presence of virulence genes of *S. enterica* strains depend on its origin. These characteristics could be used to evaluate the virulence variability of this pathogen.

P3-16 High-Throughput Screening of the Antimicrobial Activity of Protein Hydrolysates Derived from Food By-Products

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Introduction: Since foodstuffs create an environment favorable to the development of microorganisms, their handling and preservation require special attention; in this context, the intensive search for new molecules of interest against the growth of spoilage microorganisms and pathogens remains a priority to circumvent the development of resistance to conventional antimicrobials.

Purpose: This work aims to develop a new high-throughput screening method designed to generate food-derived cocktails of peptides and quickly identify active blends as well as their associated microbial targets.

Methods: This process starts with the generation of peptide pools from real waste food and biomass using different industrial proteases. The resulting mixtures were tested using a cell viability marker detected in absorbance or fluorescence to produce a fully automated routine based on a liquid-handling platform with incubators and fluorimetric plate readers. This high-throughput screening routine allowed the evaluation of 24 samples (and blanks) in 1h (plus incubation times for targets growth), which resulted in a proof of concept of 64 peptide pools (including blanks) to be screened in parallel against several microbial targets while keeping the entire procedure in sterile and controlled conditions.

Results: This new methodology allowed the screening of several samples including antimicrobial peptides, against two model microorganisms, one Gram-negative and one Gram-positive bacterium. The poster presents the results obtained during the development of this new approach and discusses the various advantages it confers and the main limitations we encountered. The ability to read fluorescence rather than just absorbance offers the possibility to overcome several challenges, the first being the sensitivity required to detect weak activities in dilute samples, as demonstrated.

Significance: The method is transposable to other pathogenic and spoilage bacteria, and allowed us during an exploratory work to identify several hydrolysates of interest that appear to be relevant for food preservation.

P3-17 Comparative Evaluation of the Efficacy of Organic Sanitizers Against *Listeria monocytogenes, Salmonella enterica, Escherichia coli* O157:H7 and Native Leafy Green 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 native leafy green 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 native leafy green 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 4.1 log 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.

P3-18 Genomic Characterization of Bacteriocins Produced by Beneficial Bacteria Isolated from Live Microbial Dietary Supplements

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Introduction: There is a growing interest in the development of alternative biological methods to control and mitigate the presence of human pathogens in the food supply. The use of bacteriocins, ribosomal synthesized antimicrobial peptides produced by microbes, could be one such alternate method. We therefore investigated our large collection of bacterial isolates from live microbial dietary supplements to identify isolates that could potentially produce bacteriocins.
Purpose: To identify the presence of bacteriocin genes associated with bacteriocin production among bacterial isolates derived from live microbial dietary supplements currently sold in the U.S. market.
Methods: Microbial contents of the products were grown on De Man, Rogosa and Sharpe (MRS) agar under different temperature and atmospheric conditions (anaerobic, microaerophilic, capnophilic and aerobic) to allow for the growth of a broad spectrum of microbial species. Purified isolates were sequenced using the Illumina platform. Whole genome sequences were identified using our in-house *K*mer database. BAGEL4, a web-based bacteriocin genome mining tool, was used to screen 2,109 whole genome sequences derived from bacterial isolates purified from 123 live microbial dietary supplements.
Results: Bacteriocin-related genes were identified in 264 isolates, which are ingredients of 92 live microbial dietary supplements. Isolates from the *Lactobacillus casei/paracasei* group were associated with the highest number of bacteriocin hits with a total of nine different bacteriocins. *Bacillus subtilis* and *Streptococcus salivarius* followed with a total of eight bacteriocins each. Carnocin_CP52 was the most prevalent bacteriocin followed by Sactipeptides, and Helveticin J. The isolates that harbor the bacteriocin genes will be screened for bacteriocin production.
Significance: Preliminary data indicate that bacteriocin gene content within live microbial dietary ingredients is significant. Beneficial microbes, components of live microbial dietary supplements, have been shown to be safe for human consumption and their antimicrobial metabolites could potentially be used as preventive tools in reducing foodborne contamination.

P3-19 Isolation of Antimicrobial-Producing Bacteria from Artisanal Cheeses and Characterization of Potentially Novel Antimicrobial Agents Produced

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✦ ***Undergraduate Student Award Entrant***

Introduction: Preservatives are often used in foods to enhance the quality and safety of a product. Traditional synthetic preservatives have a largely negative consumer perception which has created a demand for natural preservatives. Some bacteria produce antimicrobial peptides that have the potential to be natural food preservatives.
Purpose: Isolate and identify new antimicrobial-producing bacteria from artisanal cheese and characterize their antimicrobial agents.
Methods: Artisanal cheeses were acquired from small producers in Ohio. Cheese microbiota were screened for production of antimicrobials against *Listeria innocua* ATCC 33090 and *Escherichia coli* K12 as indicator strains. The microbiota were captured on cellulose microfilters and grown on suitable agar media. The filter-colony layers were removed, and the agar base was overlayed with indicator strains in soft-molten agar. Isolates that showed activity on agar media were tested against the two bacterial indicators, as well as *Candida albicans* SC5324, in 96 well plates using cell-free supernatant neutralized to pH 6. Isolates that showed activity were identified using 16S rRNA gene sequencing and their whole genomes were sequenced using Illumina MiSeq platform. Genome-based discovery of biosynthetic gene clusters associated with antimicrobial production was done using antiSMASH software.
Results: Cheese microbiota screening produced nine isolates with promising anti-Gram-negative and anti-Gram-positive activity. The antimicrobial-producing isolates grew optimally in MRS broth to OD_{600} of 1.013-1.923. Heat maps from bioassays against different targets showed diversity in antimicrobial production, indicating production of multiple compounds. Genome mining revealed 14 gene clusters, many of which encode potentially novel antimicrobial peptides, having less than 50% amino acid similarity with known bacteriocins including lactococcin, enterocin A, salivaricin, and gassericin.
Significance: The strains studied have biosynthetic gene clusters encoding novel antimicrobial peptides that may be usable in the future for food preservation.

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P3-20 Evaluation of Different Organic Acids for Controlling Multiple Foodborne Bacterial Pathogens

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❖ ***Developing Scientist Entrant***

Introduction: Evaluating and enhancing antimicrobial interventions are critical needs that might lead to better controls for the food industry.

Purpose: Here, we compared the effect of different organic acids (citric acid, malic acid, and quinic acid) on various strains of foodborne bacterial pathogens including *Campylobacter*, *Salmonella*, Shiga-toxin producing *E. coli*, and *Listeria monocytogenes* in culture and on food.

Methods: The minimum inhibitory (MIC) and bactericidal (MBC) concentrations were assessed for each organic acid. Bacterial cultures were adjusted (OD_{600} of 0.05) and incubated with different concentrations of the organic acids. 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 organic acids were also evaluated against artificially-contaminated chicken breast meat and lettuce. Each meat and lettuce sample was inoculated with ~8 log CFU/g of bacterial cocktails and immersed in solutions containing the organic acids. CFU counts were determined as described earlier.

Results: The organic acids had the lowest MICs (2.5-5.0mg/ml) and MBCs (2.5-5.0mg/ml) against *Campylobacter*. MICs and MBCs against *Salmonella* and *E. coli* were 5-10mg/ml and 10-40mg/ml, respectively. MICs and MBCs against *L. monocytogenes* were 10-20mg/ml and ≥ 20mg/ml, respectively. Reductions in *Campylobacter* and *Salmonella* loads on chicken ranged between 1.4-2.6 and 1.2-1.9 log CFU/g, respectively, using 80mg/mL of either citric acid, malic acid or quinic acid for 30 minutes. Using 160 mg/ml of the organic acids, *E. coli* and *L. monocytogenes* decreased by 1.1-2.1 and 1.1-1.9 log CFU/g on chicken, respectively. *Salmonella* counts on lettuce decreased by 1.4-2.8 log CFU/g using 40mg/ml of the organic acids, while 80mg/ml reduced *E. coli* and *Listeria* counts on lettuce by 1.2-1.5 and 1.3-2 log CFU/g, respectively.

Significance: The evaluated organic acids might not be optimal o control foodborne bacterial pathogens on meat and fresh produce.

P3-21 Decreased Vero Host-Cell Internalization of Foodborne Bacteria Using a Yeast Fermentate Extract

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Introduction: Foodborne bacterial pathogens can attach and internalize to host cells and cause severe disease illness. Natural extracts that prevent attachment and internalization can be used to prevent or alleviate illness symptoms.

Purpose: This study determined the ability of a commercial yeast fermentate (RX) to prevent host cell attachment and internalization of select Gram-positive and Gram-negative foodborne bacterial pathogens of human health concern.

Methods: Confluent Vero host cells in 6-well plates were infected with 100 µl of overnight cultures of *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Shigella flexneri* at approximately 5 to 7 log CFU/ml. Control wells contained 400 µL cell-culture (DMEM) media only, while treatment wells contained media with RX (non-cytotoxic levels of 1:20 v/v dilution) for 2 h at 37°C. Washed cells were lysed with 500 µl of cold 0.1% Triton X-100 for attachment assays along with added 10 µg/ml gentamicin for internalization assays. The attached or internalized bacteria were ten-fold serial diluted and surface spread on Tryptic Soy Agar plates. Bacteria were enumerated after incubation at 37°C for 24 to 48 h. Control (untreated) bacterial counts were compared to treated bacterial counts to determine inhibition of attachment or internalization to host cells. Each experiment was replicated thrice. Data were statistically analyzed using SAS.

Results: The RX extract at 1:20 dilution did not decrease Vero host-cell attachment of the tested Gram-positive and Gram-negative bacteria. However, after host-cell internalization for 2 h using RX, *E. coli* O157:H7 showed significant reduction ($P \leq 0.05$) of 2.9±0.75 log CFU/ml, *S.* Typhimurium and *S. flexneri* showed 1.91±0.39 and 2.02±0.10 log CFU/ml reduction, while *L. monocytogenes* and *S. aureus* showed 1.87±0.52 and 1.97±0.62 log CFU/ml reduction, respectively.

Significance: Overall, RX showed promise to prevent internalization of select foodborne pathogenic bacteria to alleviate or prevent disease symptoms.

P3-22 Antimicrobial Effect of Bacterial Cellulose Impregnated with Silver Nanoparticle Against *E. coli* O157:H7 and *Listeria monocytogenes*

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❖ ***Developing Scientist Entrant***

Introduction: Bacterial cellulose has attracted significant interest because of its potential use as a functional material in different industries. The use of bacterial cellulose impregnated with antimicrobial agents could potentially reduce microbial growth and maintain the quality and safety of foods.

Purpose: This study examined the antimicrobial efficacy of Bacterial cellulose impregnated with silver nanoparticles (AgNP) against foodborne pathogens.

Methods: Bacterial cellulose (BC) was produced by Komagataeibacter xylinus (ATCC 53524) in Hestrin-Schramm medium at 30°C for 5 days under static conditions. Silver nanoparticles were produced by using 1% Soluble starch or 5% Pullulan with 1 ml of a 100 µM $AgNO_3$ and autoclaved for 5 min at 121°C. The purified and dried BC disc was immersed in silver nanoparticles (AgNP) for 1 hour and placed on a Mueller Hinton Agar plate with *E. coli* O157:H7and *Listeria Monocytogenes*. The zone of inhibition was examined after incubating for 24 hours at 37°C. The water holding capacity of dried pure BC, and AgNP-BC was monitored by immersing them in 20 ml of distilled water for 24 hours. All the experiments were done in duplicate.

Results: The swelling ratio of BC film (6239.46 ±14.99%) was significantly lowered ($P<0.05$) after impregnated with AgNP (65.44 ± 0.17%). The water binding capacity of the BC-AgNP decreased significantly (P<0.05) compared to BC films. Bacterial Cellulose disc didn't produce any inhibition against both pathogens. However, BC-AgNP with pullulan significantly inhibited *E. coli* O157:H7 and *Listeria monocytogenes* growth by 13.5±0.354 mm and 18.5±4.243 mm, while BC-AgNP with starch inhibited bacterial growth by 11.25±0.354 mm and 13.5±1.061 mm respectively.

Significance: Bacterial Cellulose impregnated with silver nanoparticles has the potential to be used in the food industry as an active and biodegradable food packaging material.

P3-23 *Pediococcus pentosaceus,* a Strain Isolated from Kimchi with Bacteriocinogenic Properties

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Introduction: Bacteriocins are ribosomal-synthesized peptides with antimicrobial activity, produced by different groups of bacteria, including lactic acid bacteria (LAB). Most of the bacteriocins produced by LAB can be described with rather broad spectra of inhibition and they offer have been suggested for applications in food preservation and pharmaceutical sector.

Purpose: The goal of this study was to explore bacteriocin producing properties for LAB isolated from kimchi.

Poster

Methods: LAB were isolated from kimchi, obtained from the region of Pohang, Korea and identified based on physiological, biochemical and molecular methods. The promising isolate, *Pediococcus pentosaceus* 732, selected based on its spectrum of activity, was evaluated for production of bacteriocin, including stability in presence of enzymes, chemicals, pH and temperatures. Presence of bacteriocin genes in strain 732 was investigated and obtained sequences analyzed. Growth of *P. pentosaceus* 732 in different pH values and presence of ox-bile was explored. Safety properties for strain 732 were evaluated.

Results: Selected isolate 732 was identified as *P. pentosaceus.* Bactericidal effect of bacteriocin produced by strain 732 on *Listeria* spp. and *Staphylococcus* spp., was shown for actively growing and stationary cells. Similar growth and bacteriocin production were observed when *P. pentosaceus* 732 was cultured in MRS at 30ºC or 37ºC. Presence of pediocin PA1 operon on the genomic DNA was recorded based on the performed PCR analysis. Good growth for strain 732 was recorded in MRS broth with pH 5.0 to 9.0 and in absence of ox-bile or concentration below 0.8%. Strain 732 can be considered as safe for human and animal applications based on negative reaction for production of biogenic amines, lipolytic, proteolytic and γ-hemolytic activity and susceptibility to different antibiotics and growth not affected by different commercial drugs.

Significance: *P. pentosaceus* 732 shows that can be considered as potential beneficial strains associated to production of Pediocin_PA1, be safe and suitable to GIT environment.

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P3-24 Effect of Food Matrix and Treatment Time on the Effectiveness of Grape Seed Extract as an Antilisterial Treatment in Fresh Produce

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Introduction: The consumption of contaminated fruits and vegetables such as cantaloupe, apples, and celery has recently been associated with an uptick in listeriosis outbreaks. While Grape Seed Extract (GSE) has the potential to be an effective and natural solution for reducing *L. monocytogenes* contamination on a variety of food matrices, levels of its effectiveness could be affected by the food matrices.

Purpose: The goal of the study was to investigate the potential of GSE to reduce levels of *L. monocytogenes* on various fresh produce, and to determine the effect of different food matrices observe any variations on its ability to control *L. monocytogenes* in these products.

Methods: 100g of cantaloupe rinds, apples, and celery portions were spot inoculated with 100ul of *L. monocytogenes* suspension to obtain approximately 7 log CFU/100g of *L. monocytogenes*, then dipped in 100, 200, 500, 1000 µg/ml GSE/distilled water for 5 and 15 minutes. Samples were homogenized and plated in triplicate on PALCAM agar with *L. monocytogenes* counts expressed as log CFU/g. Statistical analysis was performed in Rstudio, an included Tukey's test as a post-hoc (P<0.05) and analysis of variance.

Results: The number of *L. monocytogenes* on the pieces of vegetables that were treated with GSE was significantly lower than the control samples (P<0.05). The extent of the decrease in *L. monocytogenes* levels differed, ranging from a reduction of 0.61 to 2.5 $\log_{10}$ CFU, depending on factors such as the concentration and duration of the treatment, as well as the specific type of produce involved. Our results indicate that the highest inhibition of *L. monocytogenes* was seen on apple matrix while the least inhibition was noticed on cantaloupe matrix in both 5 and 15 minutes' treatment time duration. Treatment for 15 minutes provided greater reduction in all products.

Significance: GSE is an effective natural antilisterial treatment for various types of fresh produce and the effectiveness of GSE varied among the different matrices.

P3-25 Isolation and Characterization of Bacteriophages from Wastewater Against Foodborne Pathogens and Antibiotic-Resistant Pathogens

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Introduction: Lytic bacteriophages are promising antimicrobial agents against pathogenic bacteria. Bacteriophages have strict host specificity, making them a promising option for specifically controlling pathogenic microorganisms. In addition, with the emerging of multidrug resistant pathogens, bacteriophage therapy against difficult-to-treat infections has received great attention in recent years.

Purpose: The project aimed to isolate and characterize newly isolated *E. coli* bacteriophages from a wastewater treatment plant in Arkansas, USA.

Methods: *E. coli* bacteriophages were isolated from raw sewage collected in a wastewater treatment plant using *E. coli* ATCC 25922 as the host. The activities of purified bacteriophages were tested against 7 Shiga toxin-producing *E. coli* strains (1 O157 and big six non-O157) and 13 multidrug-resistant clinical isolates from the CDC Antibiotic Resistance Isolate Bank. Bacteriophage DNA isolation was carried out using the Norgen phage isolation kit. The whole bacteriophage genomes were sequenced using Illumina NextSeq and assembled using the genome assembly pipeline from the Bacterial And Viral Bioinformatics Resource Center (https://www.bv-brc.org/).

Results: A total of 11 *E. coli* bacteriophages were isolated from 15 wastewater samples. Most of the newly isolated bacteriophages showed a broad spectrum of activity against a variety of *E. coli* strains. For example, bacteriophage EC6 was active against 10 out of 13 multidrug-resistant *E. coli* and 2 Shiga-toxin producing *E. coli* strains (O103:H11 and O145:NM). Bacteriophage EC9 showed activity against 7 out of 13 multidrug-resistant *E. coli* and 4 Shiga-toxin producing *E. coli* strains (O157:H7, O103:H11, O145:NM, and O26:H11). After genome assembly, bacteriophage EC6 was assembled into a single contig and the whole genome consists 166,767 base pairs. The closest relatives of EC6 is in the genus of *Tequatrovirus*.

Significance: The newly identified *E. coli* bacteriophages may have a potential application against pathogenic foodborne *E. coli* and multidrug-resistant clinical isolates.

P3-26 Inhibition of *Clostridium botulinum* by Antimicrobial Ingredients in a Model Meat System

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Introduction: *Clostridium botulinum* is a concern in vacuum-packed products where it can grow and produce botulinal toxin due to the favorable environment. In cured meats, sodium nitrite has been used as a gold standard preservative for *C. botulinum* control; however, alternative antimicrobials are needed for uncured meat products.

Purpose: To evaluate and compare the efficacy of lactate and acetate-based antimicrobials to delay *C. botulinum* toxicity in a model meat system.

Methods: Ground turkey formulated with five treatments (no antimicrobial, 100 ppm sodium nitrite with 250 ppm ascorbate, 0.80% Opti. Form® Powder Ace S50, 4.80% Purasal® S, and 2.50% Verdad® Opti Powder N510) were inoculated with a 10-strain cocktail of proteolytic and non-proteolytic *C. botulinum* (Types A, B, and E) at 2.50 log spores/g. Samples (25 g) were vacuum packaged, cooked to 73°C, chilled, and incubated at 7.2°C and 12.8°C for up to 20 weeks. Triplicate samples were assayed for botulinal toxin using the standard mouse bioassay. Enumeration of non-proteolytic strains at 7°C is unreliable, therefore only samples stored at 12.8°C were plated for *C. botulinum* (differential Reinforced Clostridial agar; anaerobic incubation, 30°C, 48 h).

Results: The turkey treatments had average moisture of 73.08±0.38, pH 6.31±0.02, 1.63±0.04% salt, and 0.982±0.002 water activity. Initial populations of *C. botulinum* averaged 2.11±0.48 log spores/g. Control samples without antimicrobials supported toxin production at 2 and 4 weeks when stored at 12.8 and 7.2°C, respectively. *C. botulinum* populations in control samples significantly (P<0.05) increased by 2.70 log at 2-week 12.8°C storage. In contrast, no growth or toxin production was detected in the remaining treatments at 7.2°C and 12.8°C through 20 weeks.

Significance: The application of lactate and acetate-based antimicrobials were efficacious in delaying the botulinal toxicity formation similar to sodium nitrite in ground turkey for over 20 weeks.

P3-27 Inactivation of Foodborne Pathogens with Nitric Oxide-Releasing Films

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Introduction: Nitric oxide (NO) can have antimicrobial activity against different Gram-positive and negative bacteria. Recently, different NO-releasing technologies have been evaluated for medical and industrial applications. One of those chemistries relies on the NO donor, S-nitroso-N-acetyl-D-penicillamine (SNAP), which can be blended within hydrophilic SP-60D-60 polyurethane films. The application of SNAP for reducing foodborne pathogens on surfaces had not been investigated before.

Purpose: This study was undertaken to assess the susceptibility of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Cronobacter sakazakii* to exposure to NO released by SNAP films.

Methods: Mixed strains of *E. coli* O157:H7, *L. monocytogenes,* and a *C. sakazakii* strain were grown separately, inoculated on stainless steel (SS) and high-density polyethylene coupons were dried overnight. Coupons were incubated for 24 h at room temperature in sealed polyethylene bags containing a 1 cm^2 polyurethane film containing 10% SNAP. Survival rate was determined using standard microbiological methods. Statistical analyses were performed using Student's t-test.

Results: Treatment of SS coupons with SNAP films containing approx. 8 log CFU *E. coli* O157:H7 resulted in an average reduction of 3.9 ± 0.15 CFU after 24 h, compared to controls ($P<0.05$). Similarly, inactivation of *L. monocytogenes* on SS coupons was at least 4.0 Log CFU. Generation of NO also reduced the viability of *E. coli* O157:H7 by 5.5 ± 0.26 Log CFU when cells were applied to HDPE coupons. *C. sakazakii* was also susceptible to treatment with SNAP films as their viable cell count was reduced by 5.4 log CFU on SS coupons. With lettuce leaves inoculated with *E. coli* O157:H7 and exposed to NO-releasing films viability reductions of less than 0.3 log CFU were observed ($P>0.05$).

Significance: This study provides an initial proof-of-concept that NO-releasing technologies can kill foodborne pathogens on inert surfaces.

P3-28 The Effect of Natural Compounds on *Salmonella* spp. Biofilm Formation

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Introduction: Biofilms are important contributors to contamination in the food industry. The use of natural compounds is a promising approach to control biofilm formation.

Purpose: To evaluate the effect of natural compounds on biofilm formation of *Salmonella* spp.

Methods: A biofilm producing strain of *Salmonella* sp. was selected using crystal violet assay, with *Pseudomonas aeruginosa* PA01 as reference. Minimum inhibitory concentration (MIC) of each compound and growth curves were determined using the microdilution method. Cristal violet assay was also used as a screening method to quantify biofilm formation with compounds. The compound with the best inhibition result was further investigated by the Calgary Biofilm Device to define different parameters such as: MIC and minimum bactericidal concentration (MBC) of planktonic cells, and minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC). The compound was then combined with sanitizers commonly used in food industry, using the checkerboard technique to determine the combination effect.

Results: *Salmonella enterica* serovar Montevideo 163 was selected as the best biofilm-forming strain. The MICs and sub-MIC concentrations of each compound were determined and used on the biofilm formation assays. The compounds that showed effect were quercetin, naringenin, eugenol, farnesol, resveratrol and cinnamaldehyde. However, cinnamaldehyde was the compound with the best biofilm inhibitory effect at low concentrations, without affecting microbial growth. We also obtained the results of MIC: 2.5mM; MBC: 4.0mM; MBIC: 2.5mm; MBEC: 3.5mM of cinnamaldehyde for the tested strain. The combination with sanitizers showed that benzalkonium chloride and ethanol present a synergistic effect when combined with cinnamaldehyde.

Significance: This work provides new information about the inhibitory potential of cinnamaldehyde and its applicability on the control of biofilm formation.

P3-29 Antimicrobial Efficacy of Carvacrol Against Foodborne and Food Spoilage Pathogens Biofilm on MBEC™ Biofilm Device and Polypropylene Surface

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Introduction: Biofilm may influence bacterial susceptibility to traditional disinfectants but identifying effective natural disinfectants as alternatives remains a great challenge to researchers.

Purpose: The objective of this research was to examine antibiofilm activity of carvacrol against *Pseudomonas aeruginosa* and *Listeria monocytogenes*.

Methods: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), antibiofilm activity of carvacrol, antibiofilm mobility of carvacrol on a polypropylene surface, field emission-scanning electron microscopy (FE-SEM), and confocal laser scanning microscopy (CLSM) were used to assess the viability of bacteria cells.

Results: It was found that different concentrations of carvacrol reduced the *P. aeruginosa* and *L. monocytogenes* biofilms developed on both the MBEC™ biofilm device and polypropylene surface dramatically. On the MBEC™ biofilm device, the biofilm reduction was 5.04 log CFU/peg at 0.06% carvacrol for *P. aeruginosa*, and 3.81 log CFU/peg at 0.12% carvacrol for L. monocytogenes. On the polypropylene surface, P. aeruginosa was reduced by 4.79 log CFU/cm^2 at 0.06% carvacrol, and *L. monocytogenes* was reduced by 4.62 log CFU/cm^2 at 0.12% carvacrol. Visual observations using an advanced microscopy confirmed biofilm reduction reports for both bacteria.

Significance: This study demonstrated that carvacrol has the potential use as a promising natural compound to prevent *P. aeruginosa* and *L. monocytogenes-related* infections. Carvacrol may also be used as a preservative to improve the value of food by inhibiting these foodborne pathogens in the food industry.

P3-30 Inhibitory Effect of Aqueous and Ethanolic Extracts of a Pomegranate Peel Against *Salmonella enterica* in Sprouted Nut Butter

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Introduction: A trend of consuming raw sprouted nut butter is emerging due to its high nutrient values. However, raw sprouted seeds have been reported as a causative factor in multiple enteric disease outbreaks. Pomegranate peel contains functional compounds that could be used as natural preservatives during the production of sprouted nut butter.

Purpose: This study evaluated the inhibitory effect of aqueous and ethanolic extracts of a pomegranate peel against strains of *Salmonella* Tennessee and Enteritidis in sprouted nut butter.

Methods: Raw, organic peanut and almond seeds inoculated with cells of the two *Salmonella* strains (1.16–1.97 log CFU/g) were sprouted in deionized water containing two concentrations of aqueous (total phenolics concentration 5.39 or 10.78 mg/ml) or ethanolic extract (total phenolics concentration 4.45 or 8.90 mg/ml) for 24 h at 25°C . Sprouted seeds were rinsed with sterile deionized water and dried at 45°C or 12 h. Dried seeds were then grounded

with olive oil and sea salt. *Salmonella* population was determined after each processing step using tryptic soy agar and bismuth sulfite agar. 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% confident interval.

Results: In both treatment groups containing the ethanolic extract or aqueous extract, significant ($P \leq 0.05$) *Salmonella* population reductions were observed after the nut seed sprouting and the rinse steps. However, after the drying and seasoning steps significant *Salmonella* population reductions were only observed with samples containing the ethanolic extract. The mean levels of *Salmonella* population reductions were 1.92–1.99 Log CFU/g for the ethanolic extract and 0.53–0.54 Log CFU/g for the aqueous extract.

Significance: The study suggests that ethanolic extracts of pomegranate peels have the potential to be used as natural preservatives in inhibiting the growth of *Salmonella* during the sprouted nut butter production process.

P3-31 Efficacy of Cultured Sugar and Natural Flavor Systems Against Mold in Pet Treats

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Introduction: The premium, clean-label segment of the pet industry has grown in recent years. High moisture pet treats are susceptible to mold spoilage. In order to meet these demands and control spoilage, clean-label products based on cultured sugar are developed.

Purpose: To determine the efficacy of cultured sugar natural flavor (CS-NF) and cultured sugar (CS) on inhibition of mold outgrowth in pet treats.

Methods: Chicken jerky pet treats were produced, packaged, and stored ambient post processing. Treatments were incorporated into the products during manufacturing as follows: (i) no preservative as negative control, (ii) commercial sample product, (iii) 1.5% CS-NF, and (iv) 1.4% CS. Treatments were spot inoculated in triplicate with a cocktail (2 $\log_{10}$ CFU/g) of *Eurotium herbariorum, Penicillium roqueforti,* and *Aspergillus oryzae*. Following inoculation, treats were placed in an air-tight container with a saturated salt solution to maintain the relative humidity (RH) at approximately 84%, and incubated at 21 °C. The treats were inspected daily for visual mold growth. An uninoculated control of each treatment was evaluated alongside the replicates in a separate air-tight container.

Results: The pet treats are susceptible to mold outgrowth, as shown by the inoculated negative control that was molding by day 58 (SD=14.7). On average the inoculated commercial sample product showed visible mold by day 67 (SD=24.8), whereas the average days for inoculated 1.5% CS-NF to mold was 95 days (SD=3.6), followed by inoculated 1.4% CS samples with 93 days (SD=4.6). Uninoculated commercial sample product molded by day 62, whereas samples containing 1.4% CS and 1.5% CS-NF delayed mold growth up to 70 days and 75 days, respectively.

Significance: High moisture jerky-style pet treats are susceptible to mold outgrowth. This study highlights that both CS-NF and CS solutions are effective mold control solutions for pet treats.

P3-32 Control of Spoilage Microorganisms in Salad Dressings Using Fermentation Based Solutions and Natural Plant Extracts

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Introduction: Microbial spoilage in sauces and salad dressings is mainly caused by yeast and Lactic Acid Bacteria (LAB). The combined effect of pH decrease and antimicrobial addition are usually required to reduce the risk of spoilage. Increasing consumer demands for clean label products necessitates the use of natural antimicrobials in sauces and dressings.

Purpose: Investigate the growth inhibitory effect of clean label antimicrobials against yeast and heterofermentative Lactic Acid Bacteria in a shelf-stable salad dressing over a period of five months.

Methods: A salad dressing model system was prepared by incorporating 0.1% potassium sorbate (PS), 0.7% Vinegar and Natural Plant extract A (C1), 0.7% Vinegar and Natural Plant extract B (C2), and 1.5% Fermentate (C3). Treatments were inoculated with a yeast and a LAB cocktail (*Lactobacillus plantarum, Lb. buchneri, L. brevis, Lb. fermentum, Lb. fructivorans)* to the level of 10^3 CFU/g of each target organism. A portion of each treatment remained uninoculated as the negative control (NC). Samples were analyzed in duplicate once a week for 4 weeks and monthly up to five months for each variable to monitor the yeast and LAB counts.

Results: Yeast and LAB counts in uninoculated samples remained below detection limit (<1 log CFU/g) during the study. Sampling was discontinued for NC after 21 days as yeast and LAB counts exceeded 6 log CFU/g. LAB population decreased below detection limit (<1 log CFU/g) within 7 days in C1, C2, C3 and PS samples ($P<0.05$). The yeast population decreased to levels <1 log CFU/g within 14 days ($P<0.05$) in samples containing all C1, C2, and C3. Yeast population in PS decreased below detection limit (<1 log CFU/g) within 7 days.

Significance: All clean label systems were shown to control yeast and LAB outgrowth in salad dressing for over five months storage period.

P3-33 Antifungal and Aflatoxigenic Activities of Clove Oil and Eugenol Against *Aspergillus Flavus* in Georgia Peanuts

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Introduction: *Aspergillus flavus* is a serious problem in Georgia peanuts. Our study reported clove oil as an eco-friendly solution to peanut contamination and aflatoxin B1 (AFB1), however, it is important to compare clove and its active ingredient, eugenol, against *A. flavus*.

Purpose: This study evaluated the antifungal and anti-aflatoxigenic effect of clove oil and eugenol, against *A. flavus* in peanuts.

Methods: The Minimum Inhibitory Concentration and Minimal Fungicidal Concentration were determined by standard methods. Known volumes of clove oil and eugenol were incorporated, separately, into PDA plates, with 0.5% of Tween 20 to make 500–2500ppm. 10μl conidial suspension of 7-day old culture was inoculated at the center of plates and incubated at 28±2◦C for 7 days. Mycelial inhibition (%) = [(*dc* − *dt)/dc*] × 100 (*dc,* colony diameter untreated & *dt,* colony diameter in treated samples). Purification of AFB1 was done by HPLC/UV, using thirty grams of each sample. AFB1 was identified by retention times based on AFB1 reference standard. Data analysis was done by using statistical software SPSS for windows version 10.0.1 and ANOVA were used to determine differences between treatments with significance levels set at $P=0.05$.

Results: Increase in clove EO or eugenol, increased zone of inhibition compared to control. At 500ppm, a 90% inhibition of mycelial growth was observed compared to 100% at 2000ppm with both EOs. The UV spectrum of AFB1 standard was identical to clove EO and eugenol-treated mycelia, both at retention times 5.158 min. At 500ppm, a 15% decrease in AFB1 was noted compared to 60% at 2000ppm, establishing a correlation between oil concentrations and AFB1.

Significance: Although there was no significant difference between impact of clove oil or its active ingredient, our findings provide evidence of potential applicability of either oil as an eco-friendly alternative to synthetic fungicides against *A. flavus* in peanuts.

P3-34 Microbiota Characterization and Shelf Life Extension of Plant-Based Meat

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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, spoilage microorganisms are poorly characterized in food, including in plant-based meat, because of their diversity and the deficiency of specific selective media for isolation. Microbial spoilage characterization enables the processors to identify 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 meat. Additionally, by performing next-generation sequencing, investigate diverse growth patterns of microbial ecology in plant-based meat.

Methods: Plant-based patties were prepared using soy or 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, lactic acid bacteria, and yeast or mold population. Additionally, 16S rRNA gene profiling was performed from sample homogenates for characterizing spoilage microbiota. The experiments were repeated twice with duplicate samples (n=4).

Results: Extensive growth of spoilage microbes was observed in control for both types of patties. The microbial count for aerobic bacteria, lactic acid bacteria, and yeast or mold reached 7 $\log_{10}$ for control patties within 7-10 days and spoiled the patties. However, DuraShield™ suppressed the growth of aerobic bacteria, lactic acid bacteria (in soy- and- pea-based patties; $P<0.05$), yeast, and molds (in pea patties; $P<0.05$) until day 21 and maintained microbial counts below 6 $\log_{10}$ CFU/g in patties. Additionally, 16S rRNA gene profiling resulted in differential distribution of specific microbiota between control and test groups.

Significance: DuraShield™ successfully controls spoilage in plant-based patties and extends the shelf life to additional 10 days compared to the control.

P3-35 Assessment of Efficacy of Smoke Systems on Meat Product Shelf Life and Food Safety

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Introduction: Smoke technology offers multiple benefits such as flavor, color, preservation impact as well as positive product characteristics. Market trends required clean label solutions for meat shelf-life and food safety extension.

Purpose: To assess the in-vitro minimum inhibitory concentrations (MIC) of smoke fractions against spoilage and pathogenic micro-organisms.

Methods: Varying concentrations 0.5, 1, 1.5, 3, and 4% of smoke products (Zesti List A Smoke Clear, Cloud S5, List A Smoke Clear M, Red Arrow Smoke EZ) and blank (no smoke fractions/broth) were prepared in De Man, Rogosa and Sharpe and brain infusion heart broth. Samples at pH 6.0 were aliquoted into 100-well honeycomb with inoculums of spoilage bacteria such as *Lactobacillus sakei* , *Lactobacillus planatarum*, *Leuconostoc mesenteroides* , *Carnobacterium divergens* , *Pseudomonas fluorescens* and pathogenic bacteria such as *Listeria monocytogenes* (serotype 1/2a, 1/2b, 4a and 4b) and *Staphylococcus aureus* (6 log CFU/g). The plates were placed in the Bioscreen-C at 37°C to generate growth curves. Modified Gompertz equation was used to determine the maximum growth rate (μmax; d^{-1}) which was further used to determine the MIC. The MIC was defined as the lowest concentration at which no-growth occurred. Data was analyzed using one-way ANNOVA ($P<0.05$).

Results: All smoke products tested, exhibited a similar inhibition pattern. Against pathogenic bacteria such as *L. monocytogenes* and *S. aureus,* all the smoke products at 0.5% exhibited significantly ($P<0.05$) reduced μmax of 0.31-0.42 d^{-1} compared to blank (*S. aureus*; 3.08 d^{-1} and *L. monocytogenes*; 3.36 d^{-1}). Smoke products at concentrations of 1% and above exhibited no growth. Similar trends were observed for *L. sakei, P. fluorescens,* and *C. divergens.* For *L. planatarum*, smoke products exhibited highest MIC of 3%.

Significance: The study highlights the antimicrobial efficacy of smoke systems against shelf life and food safety related meat microorganisms and provides industry with a natural multi-functional ingredient.

P3-36 Vinegar as a Secondary Inhibitor to Control Outgrowth of *Listeria monocytogenes* and Extend Shelf Life by Inhibiting Mold Growth in Shredded Cheese

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Introduction: Potential temperature abuse and inadequate handling by consumers can lead microbial contamination and increase the risk of illness. *Listeria monocytogenes* is a big challenge in cheese production. Natamycin and Nisin compounds are used widely in the cheese industry to increase the shelf life of shredded cheeses as mold control agents.

Purpose: To evaluate the antimicrobial efficacy of dried buffered vinegar to inhibit the growth of *Listeria monocytogenes* and mold in shredded cheeses.

Methods: Whole blocks of cheddar and mozzarella cheese were shredded. Shredded cheese samples were treated with buffered vinegars; and Nisin at 100 ppm (TC1- cheddar) and Natamycin at 20 ppm (TM1- mozzarella) as positive control. Treatments included buffered vinegar 1.0% for cheddar (TC2) and 0.75% for mozzarella (TM2); buffered vinegar 2 rates 0.5% for Cheddar (TC3) and 0.25% for mozzarella (TM3). A five- strain (ATCC 19111, 19112, 19115, 19118 and 13932) cold-adapted cocktail of *Listeria monocytogenes* and isolated indigenous strain of mold were used individually to inoculate the samples treated with antimicrobials at a target inoculum rate of 2–3 log CFU/g. After the microbial attachment, samples were heat sealed and stored at 39±1.5°F for 40 days and enumerated for *L. monocytogenes* and mold at days 13, 26, 33 and 40 using selective media. Samples were taken from the same respective packs to mimic the domestic storage and handling conditions.

Results: The results indicate that the buffered vinegars 1 and 2 were effective and on par with Natamycin and Nisin in controlling outgrowth of *Listeria monocytogenes* and reducing the mold populations in both the cheese samples with $P>0.1$ in both ANOVA and *t*-test.

Significance: Buffered vinegars were effective in controlling *Listeria monocytogenes* and mold growth; extending shelf-life of shredded cheeses; improving safety in domestic handling environment.

P3-37 Extending Shelf Life of Salad Dressings Using Clean-Label Antimicrobials

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Introduction: The shelf-life of dairy-based salad dressing is expected to be at least 3 to 6 months under the refrigerated condition. The general product failure is microbial spoilage and off-flavors development. Traditional antimicrobials such as Natamycin, Nisin and some organic acids are used to extend the shelf life commercially. There is an increasing need in the market to utilize naturally derived ingredients on formulations while maintaining quality and safety and minimizing waste.

Purpose: To evaluate the efficacy of buffered vinegar in controlling the growth of lactic acid bacteria (LAB), and yeast and mold (YM) in dairy-based salad dressings.

Methods: Commercially formulated Ranch, Bleu cheese and Caesar salad dressings treated with and without buffered vinegar with pH 3.8±0.3 at 1.45% usage rate were stored at 39±1.5°F and used for shelf-life study. The pH of the samples was measured in triplicate at 24±1°C on each sampling day 1 to 120 for 6 sampling point days. Samples were tested in triplicate as per AOAC standard methods for Aerobic Plate Count, Lactic Acid Bacteria and Yeast and Mold Count at each sampling point.

Results: Increase in pH was observed during the storage period for all the samples. The shelf life results indicated that the buffered vinegar at 1.45% usage rate was effective in controlling the growth of YM in all three treated salad dressings throughout the shelf-life period with $P<0.05$ (*t*-test) compared to the control products. Buffered vinegar inhibited the growth of spoilage LAB in the salad dressings and its effect was on par with the control sample containing commercial antimicrobials.

Significance: Buffered vinegar was effective in controlling the growth of YM and LAB in all three salad dressings and comparable to the commercially used antimicrobials like Natamycin and extending the shelf-life.

P3-38 Investigating a Multi Hurdle Antimicrobial Application to Improve Safety and Shelf Life of Ready-to-Eat Turkey (RTE) Turkey and Ham

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Introduction: *Listeria monocytogenes* can grow at refrigeration temperature and even in low oxygen environment like vacuum package products; therefore, it commonly occurs in RTE products. There are several antimicrobials available to control *Listeria monocytogenes* growth as a secondary inhibitor and they either be added to product formulation or sprayed as surface applications.

Purpose: To evaluate the antimicrobial efficacy of dried buffered vinegar and lauric arginate (LA) to inhibit the growth of *Listeria monocytogenes* and extend the shelf life of RTE ham and turkey.

Methods: Samples of oven roasted turkey and honey maple ham, treated with dried buffered vinegar, which was added to the product formulation, sprayed with LA and untreated control (no LA and dried vinegar) were used. A five-strain (ATCC 19111, 19112, 19115, 19118, 13932) cold-adapted cocktail of *Listeria monocytogenes* was used to inoculate the samples at a target inoculum rate of 2 to 3 log CFU/g. After inoculum attachment, samples were MAP packaged with 70:30, Nitrogen: Carbon dioxide, gas mix. All samples were stored at 39 ±1.5 °F for 120 days. Samples were enumerated for *L. monocytogenes* and other spoilage organisms such as total aerobic bacteria, lactic acid bacteria, yeast, and mold.

Results: Results showed that the buffered vinegar was effectively controlling the outgrowth of *Listeria monocytogenes* in both RTE ham and turkey. Both LA treated and control showed overgrowth of *Listeria monocytogenes* at the sampling day of 31 and 34, respectively. *t*-test showed no significance with $p > 0.3$ for ham and turkey for LA treated and control samples. LA and buffered vinegar were significant with $p < 0.05$ in controlling the growth of *Listeria monocytogenes* in turkey and ham. No growth of APC, lactic acid bacteria, yeast, and mold were observed in shelf-life study.

Significance: Buffered vinegar alone was effective in controlling *Listeria monocytogenes and* extending shelf-life of RTE meat compared to LA.

P3-39 Developing a Bacteriophage-Based Biological Control System for Stem Gall Disease in Highbush Blueberry (*Vaccinium corymbosum*)

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Introduction: Stem gall disease, caused by *Rhizobium radiobacter*, forms tumors on blueberry stems that prevent transpiration and transport of water and nutrients; as a result, infected plants display yield loss, decline and death over the years. Bacteriophages or phages are viruses that specifically target host bacteria by causing cell lysis which can be used in agriculture to control bacterial pathogens.

Purpose: Our objectives are to develop and optimize phage isolation methods from farm environment, and to examine the ability of phages to lyse and prevent cell multiplication of *R. radiobacter in-vitro* and in soil.

Methods: Phages were isolated from various environmental sources such as soil, drainage ditch, and sewage influents. A total of 72 phages were isolated from sewage influent, and screened for their inhibitory ability against *Rhizobium* spp. The latent period and burst size of these chosen phages were determined using a single-step growth curve.

Results: Among the phages examined, the top five candidates, IC12, IG49, AN01, LG08 and LG11 were able to inactivate 21, 16, 22, 25 and 22 out of 28 *Rhizobium* strains, respectively. These lytic phages had similar latent period ranging from 90 to 110 minutes and the burst size ranging from 8 to 15 phages per infected cell except for IG49, having a burst size of 33 phages per infected cell. Therefore, IC12 was chosen as a single phage to test in a soil-based system artificially inoculated with *R. radiobacter* strain M11 with a multiplicity of infection (MOI) of 100 PFU/CFU. A significant ($P<0.05$) reduction of 6, 4 and 2 log CFU/ml was observed during the incubation of days 1, 2 and 3, respectively, when compared to untreated soil.

Significance: These results demonstrated that phages serve as a promising biocontrol agent against *R. radiobacter* and can be used for preventing yield loss in the early food production chain.

P3-40 Development of a Plant-Derived Extract Mixture to Replace Synthetic Preservatives for Production of Clean Label Products

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Introduction: Large amounts of additives are used during the processing of meat products to maintain their quality and shelf life. With the growing interest in healthy eating, natural plant-based additives are being used as alternatives to synthetic additives.

Purpose: In this study, we developed a plant-derived extracts mixture to replace synthetic preservatives for production of clean label products.

Methods: One-hundred plant-derived natural extracts were tested antimicrobial activities by analyzing agar diffusion assay, and the extracts showing antimicrobial activity were subjected to minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) analysis. For each selected extract, the optimal blending ratio was derived using Graphic Expert program, and the optimal mixture was subjected to MIC and MBC analysis. Finally, we confirmed its antimicrobial activity, lipid oxidation and preservation (changes of pH, color, thiobarbituric acid reactive substances) on sausages formulated with the optimal mixtures.

Results: The natural extract mixtures exhibited different antibacterial activities, depending on the combination. Compared to grapefruit seed extract, a mixture of natural extracts extracted with ethanol (M4) reduced the *Escherichia coli* content by more than 99.9% after 8 days of storage and slowed the growth of *L. monocytogenes* and *Salmonella* spp. by more than 80% at 14 days. Compared to untreated (NC) and grapefruit extract (PC)-treated sausages, sausages treated with the natural extract mixtures showed a significant decrease in the CIE L* and an increase in the CIE a* and CIE b* ($P<0.05$). *The pH value was significantly lower in sausages with natural extract mixtures than in NC and PC sausages (*$P< 0.05$). The natural plant extract mixtures significantly prevented lipid oxidation ($P< 0.05$).

Significance: In sum, different types of natural extract mixtures have a synergistic effect when used together, suggesting that natural preservatives can generally inhibit the growth of microorganisms and oxidation of processed meats.

P3-41 Efficacy of Chitosan on Quality and Shelf Life of Goat Meat Patties

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❖ ***Developing Scientist Entrant***

Introduction: In the United States, an increase in the ethnic populations has led to the demand for an alternative protein source such as goat meat. With the consumer demand for natural preservatives in their foods, chitosan can be used as a natural preservative for goat meat. The multi-functional properties of chitosan, antimicrobial and antioxidant, make it an effective solution to replace current synthetic methods of meat quality and shelf-life.

Purpose: To assess the effectiveness of chitosan on the quality and shelf-life of goat meat patties stored at 4°C for 16 days.

Methods: The *longissimus thoracis* muscles of goats fed 12% and 16% sweet feeds were trimmed, ground, and assigned to four treatments: Control (12%), 2.5% Chitosan (12%), Control (16%) and 2.5%- Chitosan (16%) and formed into patties. The aerobically packaged patties were stored at 4±1 °C for 16 days. The physicochemical (pH, color, cook loss and NIR) and microbiological (mesophilic count) properties were replicated three times and assessed intermittently throughout storage.

Poster

Results: Chitosan increased the pH of the goat meat patties; the 16% chitosan treated patties had a significantly higher (P<0.05) pH of 6.7 than the other treatments (~6.4). Similar cook loss (71% - 90%) was observed for all treatments. The patties with 2.5% chitosan (12% and 16%) had different (P<0.05) NIR (fat, moisture, protein, and collagen) results compared to the control treatments. The chitosan treated patties had lower L, a* and b* values compared to the controls. Overall, the chitosan treatments (12%-6 log CFU/g, 16%-8 log CFU/g) did not improve (P>0.05) the microbiological profile when compared to the control (12%-5 log CFU/g, 16%-7 log CFU/g).

Significance: This research provides a practical method in application of chitosan coatings on preservation of goat meat to improve the safety, quality and extend the shelf life.

P3-42 Efficacy of Cultured Sugar and Vinegar Systems Against Spoilage Bacteria in Plant-Based Meat Analogue

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Introduction: Plant-based meat category continues to grow well globally. With continued demand as a meat substitute, health is a main driver to consumers, making clean-label ingredients desirable.

Purpose: To evaluate the antimicrobial efficacy of fermentate, vinegar (BV), and organic acids on plant-based chicken (PBC) analogue.

Methods: Separate studies were conducted. In study 1, PBC was produced with three different formulations: (i) 0.75% vinegar for positive control (PC), (ii) 0.75% vinegar and 1% fermentate A and (iii) 0.75% vinegar and 2% fermentate B. In study 2, PBC was produced with five different formulations: (i) PC with no antimicrobial, (ii) 2.25% BV and fermentate B, (iii-v) 2.25% BV and fermentate B with 0.1, 0.2, and 0.4% organic acids, respectively. Each formulation was inoculated with a cocktail (~3 $\log_{10}$ CFU/g) of *Lactococcus lactis*, *Lactococcus* sp., *Enterococcus faecium*, *Aerococcus* sp., and *Carnobacterium maltaromaticum*, then homogenized and stored at 4°C and 7°C. Samples were stomached and plated in duplicates onto deMan, Rogosa, Sharpe for enumeration lactic acid bacteria (incubated anaerobically) until spoilage level achieved (~7 log CFU/g). Data was analyzed using one-way ANNOVA.

Results: In study 1, at 4°C all treatments formulated with either vinegar and fermentate A or fermentate B significantly (P<0.05) controlled growth up to 59 days storage compared to PC which grew until ~5 log CFU/g. On day-59, 0.75% vinegar and 2% fermentate B treatment exhibited fewer populations (~1.85 log CFU/g). At 7°C, 0.75% vinegar and 2% fermentate B treatment was most effective with an additional 7 days of shelf-life over PC (spoilage at day-12). In study 2, at both temperatures it was observed formulation with 0.4% organic acids (treatment v) was most effective (54 days of shelf-life) compared to PC which showed spoilage at day-13 (4°C) and day-5 (7°C).

Significance: The research substantiates the antimicrobial efficacy of clean-label fermentate and vinegar-based solutions against PBC spoilage bacteria.

P3-43 Combinatorial Supplementation of *Moringa* o*leifera* Leaf Extract and Citric Acid Improves the Quality of a Fruit-Vegetable Blend

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Introduction: The dietary invaluableness of fruit-vegetable juice blends (FVJB) cannot be overemphasized in the management of chronic diseases. However, FVJB is prone to quality deterioration and the use of chemical preservatives is not an option. Thus, dietary supplements with preservative effects are in high demand for FVJB quality improvements.

Purpose: Therefore, this study aimed at assessing the effects of *Moringa oleifera* leaf extract (MOLE) and citric acid in enhancing the quality of FVJB (apples, pears, spinach, and cucumber).

Methods: FVJB produced and preserved with different concentrations of MOLE and citric acid was subjected to microbial, proximate composition, physicochemical, and phytochemical analyses using approved methods.

Results: The FVJB supplemented with 1% citric acid plus 5% MOLE (A), 1% citric acid plus 10% MOLE (B), and 1% citric acid plus 15% MOLE (C) showed lower microbial counts with 1.26±0.03 log CFU/ml, 2.51±0.09 log CFU/ml, and 0 log CFU/ml, respectively, compared with the control (12.59±0.81 log CFU/ml), without citric acid and MOLE. The physicochemical and phytochemical analysis showed a significant improvement (P<0.05) in pH (3.02), total soluble solids (14.5%), total titratable acidity (2.11), and total phenolic content (125±0.03 mg GAE/ml) for sample C. The total flavonoid content of FVJB C increased significantly (P<0.05) compared with the control (100±0.02 µg/ml, 45±1.0 µg/ml, respectively). A similar trend was noticed in the proximate composition of the FVJB with sample C having the highest (P<0.05) composition.

Significance: MOLE and citric acid demonstrated significant enhancements in the microbial, proximate composition, physicochemical, and phytochemical characteristics of the FVJB. Their incorporation proved to be an effective alternative to chemical preservatives or additives.

P3-44 Cold Plasma and Organic Acid Treatment Combination Enhances Inactivation of *Salmonella* Bacteria on Tomato Stem Scar Surfaces

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Introduction: Produce industry and research institutions are trying to come up with ways to reduce bacterial populations on fruits and vegetables.

Purpose: In this study, we investigated antimicrobial efficacy of organic acid solution combined with cold plasma treatment in reducing aerobic mesophilic bacteria and *Salmonella* populations inoculated on tomatoes stem scar surface.

Methods: Tomatoes were spot inoculated or by total submersion in 107 CFU/ml *Salmonella* inoculum for 3 min to achieve an average of 5.6±0.14 log CFU/ g and 3.9±0.12 log CFU/g, respectively. All inoculated tomatoes were treated separately with antimicrobial solution, cold plasma, and a combination of the two for 30 s, 60 s, 120 s, 180 s and 360 s. Results calculated were reported as log CFU/g All experiments were repeated three times with duplicate samples analyzed. Colonies enumerated on selective and non selective agar were reported as log CFU/g of tomatoes and the data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS; SAS Institute, Cary, NC)

Results: Separate treatments of each for 120 s led to a significant inactivation of bacterial populations. Treatments with organic acid alone for 180 s led to 1.9 log CFU/g inactivation of aerobic mesophilic bacteria and 1.6 log CFU/g for cold plasma, while both treatments led to <0.1 CFU/g for yeast, and mold. Treatment combination led to significant (p<0.05) inactivation of bacteria than individual treatment. Example, treatments for 120 s led to 2.2 and 1.9 log CFU/g inactivation of *Salmonella* bacteria, respectively on spot and dip inoculated tomatoes and reduced the aerobic mesophilic bacteria populations to <0.3 CFU/g.

Significance: This result suggests that a combination treatment for ≥120s to ≤180 s is recommended for a higher inactivation of *Salmonella* bacteria on tomatoes stem scar surfaces to improve the microbial safety

Poster

P3-45 A Novel Photothermal Nano-Clay Carrier Preserving Essential Oils for Photo-Triggered Bacterial Inactivation

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Developing Scientist Entrant

Introduction: Due to their broad-spectrum antimicrobial activity and low bacteria resistance induction, natural antimicrobial materials have become promising potential alternatives to traditional agents, but still have their limitations in practical applications.
Purpose: The objective is to synthesize a food-contact grade photothermal nano-clay carrier to preserve essential oils and to achieve effective antimicrobial effects through light controls.
Methods: The photothermal nano-clay was prepared to provide loading space for carvacrol (CA) through dispersed monolayer montmorillonite/polydopamine composites by hydrogen bonding and π-type interactions. The cross-linking of alginate with calcium ions was employed to form a tight shell layer for preserving the CA and to dynamically release the CA vapor through the photothermal effect to achieve the antimicrobial effect. After the preparation, scanning electron microscopy was utilized to observe the microstructure and morphology of the nanocomposites, and high-performance liquid chromatography was used to determine the CA loading capacity. The photothermal conversion efficiency of the nanocomposite was measured under near-infrared laser (NIR) irradiation at the wavelength of 808 nm and a power of 3.0 W/cm^2. The antibacterial efficiency of *E. coli* and *S. aureus* (from 10^5 CFU/ml) was measured by irradiating the nanocomposite of 5 mm diameter in a 1 L jar under the NIR for 5 min.
Results: SEM results showed a uniform lamellar structure with porous structure in the cross section. The loading efficiency of CA in three nanoclay tablets of about 7 mg was 17.55±1.07% and the encapsulation efficiency was 38.46±2.35%. Under the NIR, the surface temperature increased to above 80°C within 30 s. The reduction was not significant (<1 log), but the antibacterial efficiency against *Escherichia coli* and *Staphylococcus aureus* reached 38.7% and 48.4% compared to control (P<0.05).
Significance: This new photothermal-controlled antimicrobial material shows the potential for expanding practical applications of essential oils as natural antibacterial agents.

P3-46 Impact of Surface Color on the Efficacy of Antimicrobial Blue Light Against *L. monocytogenes*

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Developing Scientist Entrant

Introduction: High density polyethylene (HDPE) is a common food contact surface. *Listeria monocytogenes* can attach to plastics and persist on food contact surfaces. Antimicrobial blue light technology (aBL) has been investigated as a novel intervention for the control of *L. monocytogenes*.
Purpose: This study evaluated the efficacy of aBL in reducing *L. monocytogenes* population on different colored HDPE plastic coupons.
Methods: Sterile HDPE coupons (8.6×5.3 cm) of colors red, blue, green, white, and black were inoculated with 8.5 log CFU/cm^2, of a 5-strain cocktail of *L. monocytogenes.* Coupons were exposed to aBL (405 nm) at a light intensity of 130.68 and 522.72 J/cm^2 for 1 h and 4 h. On completion of the treatment, coupons were sonicated for 5 min, and enumerated on tryptic soy agar with 0.6% yeast extract and Rapid *L. mono*® plates after incubation at 37°C for 48 h. Inoculated coupons kept at dark and exposed to a white, fluorescent tube light (0.2 J/cm^2) were considered as positive and negative controls. Experiments were conducted as three biological and three technical replicates. Statistical significances were determined using ANOVA.
Results: aBL exposure for 1 h resulted in LM reduction on blue, black and red coupons by 2.13±0.5, 1.97±0.56, 1.86±0.56 log CFU/cm^2 respectively. aBL treatment for 4 h resulted in LM reduction on blue, black and red coupons by 2.85±0.20, 2.13±0.25, 2.12±0.16 log CFU/cm^2 respectively. *L. monocytogenes* population on green coupons after 1 and 4 h were 1.45±0.41 and 2.04±0.14 log CFU/cm^2. Similarly, lowered reduction was observed on white coupons after 1 and 4 h with reductions of 1.65±0.16 and 2.05±0.37 log CFU/cm^2. Color of the coupon had a significant impact on the efficacy of the aBL treatment against *L. monocytogenes* (P≤0.05).
Significance: The study indicates that material color is an important factor contributing to efficacy of aBL treatment.

P3-47 Comparison of Antimicrobial Efficacy of Plasma-Activated Water Against *Listeria monocytogenes* Grown in the Planktonic and Biofilm Modes

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Introduction: Biofilms confer resident bacteria with increased tolerance to environmental stress, leading to biofouling and incomplete sterilization. Plasma-activated water (PAW) is a novel method for decontamination, but its effectiveness against bacteria in different life modes is yet to be determined.
Purpose: This study aimed to determine the antimicrobial efficacy of PAW against planktonic and biofilm cells of a common foodborne pathogen, *Listeria monocytogenes*.
Methods: PAW was prepared with double distilled water activated using cold plasma for 5, 10, and 15 minutes. PAW properties (pH and oxidation-reduction potential (ORP) values) were determined for each activation time. *L. monocytogenes* culture at the stationary phase and one-day-old biofilms were treated with PAW for 6 and 24 hours. Total plate count and crystal violet assay were used to evaluate bacterial viability and biofilm biomass, respectively. Statistical significance was determined using one-way ANOVA with Tukey's tests (P<0.05).
Results: PAW with longer activation times had lower pH values and higher ORP values. For *L. monocytogenes* planktonic cells, 10- and 15-min PAW treatments caused a more than six-log reduction in total bacterial counts after 6 hours. However, in the test against *L. monocytogenes* biofilms, the biofilm biomass increased 1.5 times in the presence of 10- and 15-min PAW after 24 hours compared to no PAW treatment. Additionally, viable bacteria dispersed from the biofilm were detected after 24-hr PAW treatment, regardless of the plasma activation time. Overall, the results indicated that treatment of PAW with a pH of 3 and an ORP level above 200 mV was effective in inactivating *L. monocytogenes* in the planktonic but not biofilm mode.
Significance: PAW treatment shows bactericidal effects on planktonic *L. monocytogenes*; however, insufficient PAW treatment can promote biofilm formation of *L. monocytogenes*. Further studies on responses of *L. monocytogenes* biofilm cells to PAW will shed light on preventing *L. monocytogenes* from developing stress tolerance.

P3-48 Synthesis of Carboxymethyl Cellulose Capped Zinc Oxide Nanoparticles and Its Antimicrobial Efficacy

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Developing Scientist Entrant

Introduction: Zinc oxide (ZnO) has been widely employed in the food industry as a nutrient enhancement and antimicrobial agent. One of the main drawbacks that hinder its application is the tendency to aggregate.
Purpose: To determine the antimicrobial activity against two microbial strains - *Listeria monocytogenes* and *Escherichia coli.* To investigate the antibacterial mechanism of ZnO-CMC nanoparticles.

Methods: Experimentally, Zn^{2+} ions were crosslinked with carboxymethyl cellulose (CMC) through electrostatic interactions followed by generation of CMC-capped ZnO (ZnO-CMC) nanoparticles through mild heating process. ZnO-CMC dispersions were prepared by hydrating ZnO-CMC powders in DI water with or without sodium acetate.

Results: Morphological studies based on scanning electron microscope and atomic force microscope confirmed that CMC assisted to control and inhibit agglomeration of ZnO nanoparticles, leading to a uniform size distribution in the range of 50-80 nm. As to the antimicrobial function, sodium acetate was employed to further facilitate dispersion of ZnO-CMC in the aqueous phase. Uniform and small particles were observed in sodium acetate dispersed ZnO-CMC due to the formation of a double layer on nanoparticle surface through positive and negative charged ionic clusters in sodium acetate. The antimicrobial capacity was evaluated against two microbial strains - *Listeria monocytogenes* and *Escherichia coli*. Compared with commercial ZnO and ZnO-CMC, sodium acetate dispersed ZnO-CMC exhibited the strongest antimicrobial activities based on the determinations of minimum inhibitory concentration and minimum bactericidal concentration. The antimicrobial mechanism was considered to arise from the rupture of cell walls, the occurrence of cytolysis, and ROS production.

Significance: Overall, the current study revealed that sodium acetate dispersed ZnO-CMC holds great potential to be applied in the food industry as a food preservation agent.

P3-49 Novel Antimicrobial N-Halamine Surface Coating Prolongs the Antimicrobial Effect of Commercial Bleach-Based Disinfectant in Food Processing Settings

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Introduction: One of the major efforts to ensure food safety is to efficiently apply disinfectants in food processing facilities. However, most current commercial disinfectants are not durable or long lasting, which leaves food safety concerns. The antimicrobial N-halamine surface coating innovation extends the durability of antimicrobial efficacy of chlorine-based disinfectants.

Purpose: To evaluate the antimicrobial efficacy of N-halamine surface coating against *S. aureus* on a stainless-steel food processing machine.

Methods: N-halamine coatings were sprayed with a Biomist spray system onto vertical stainless-steel surfaces of a sausage cutting machine. Following the cleaning routine, diluted cleaner and disinfectant were sprayed onto the vertical stainless-steel surface daily. On the 3rd day, *Staphylococcus aureus* ATCC 6538 diluted with PBS with or without 5% fetal bovine serum (FBS) was inoculated onto vertical stainless-steel surfaces. After drying for 2 hours in the environment of RH at 23%~ 48%, at room temperature, residual *S. aureus* was harvested with 3M quick swab, resuspended in the letheen broth, plated onto 3M staph express Petri film. The surfaces not coated with N-halamine served as negative control. Three replicates were plated for each treatment. Three individual experiments were performed. Statistical analysis was performed with ANOVA.

Results: In the presence of 5% FBS, (4.42±0.16) log (CFU/100 cm^2) of *S. aureus* was recovered from uncoated surfaces, (1.43±0.06) log (CFU/100 cm^2) reduction was achieved on surfaces coated with N-halamine. In the absence of 5% FBS, (3.19±0.51) log (CFU/100 cm^2) of *S. aureus* was recovered from uncoated surfaces, (0.96±0.44) log (CFU/100 cm^2) reduction was achieved on surfaces coated with N-halamine.

Significance: Compared with the noncoated vertical surface, the N-halamine surface coating can maintain and enhance the antimicrobial efficacy of commercial chlorine-based disinfectant and reduce *S. aureus* by ~ 1.5 log (CFU/100 cm^2) and ~0.96 log (CFU/100 cm^2) with and without 5% FBS soil load respectively.

P3-50 New Antimicrobial Processing Aid for Listeria Control in RTE

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Introduction: Food contamination by pathogens such as *Listeria spp.* remain very frequent and the recall they cause are costly ($10M to $30M) in addition to the negative impact on the company's brand image.

Purpose: The objective is to use antimicrobial peptides, in this case a bacteriocin (Pediocin PA-1 M31L), in order to provide an inhibitory solution specific to *Listeria spp.* without affecting the organoleptic qualities of the food.

Methods: Pediocin PA-1 ML31 is obtained by solid-phase peptide synthesis. To characterize its activity, soft agar plates and determination of the minimal inhibitory concentration (MIC) against *Listeria monocytogenes* were used to determine its inhibitory effect. Challenge tests were performed to validate its activity and to determine the concentration of application by reproducing a bacterial contamination of different food. To ensure the safety, degradation on food (HPLC MS/MS) and cytotoxicity (in vitro assay) experiments were performed.

Results: Pediocin PA-1 M31L is a more stable and efficient peptide than the native version as it is less inclined to oxydation. In fact, the peptide version has a MIC of 37,8nM that is stable throughout more than a year. Once applied on food, it can reduce the initial contamination by 2 to 3Log with a concentration of 150ppb. Finally, the degradation experiments shows that the only by-product are amino acids and that cells expose to Pediocin PA-1 M31L have a level of viability between 95-100%.

Significance: Pediocin PA-1 M31L is a safer alternative due to its natural peptidic composition that consist of natural amino acid as opposed to the oxydative action of chemical disinfectants and preservatives. It is the first peptide to be approved in 2020 by Health Canada and the CFIA as an antimicrobial processing aid making it available for commercial use without having to label it in the list of ingredients.

P3-51 Efficacy of Peracetic Acid (PAA) in Combination with a PAA Booster Against Bacterial Biofilm and Endospores

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Introduction: Peracetic acid (PAA) is one of the most commonly used sanitizer/disinfectants in the food industry due to its efficacy, low cost, lack of toxic residues and easy application. Nevertheless, when used alone, undesirably high levels of PAA may be needed to achieve activity against difficult to control microbial targets such as biofilm and endospores.

Purpose: A formulation containing a mixture of organic acids, chelants, surfactants and biodispersant was developed and evaluated for its ability to enhance PAA performance against bacterial biofilm and endospores.

Methods: The Minimum Biofilm Eradication Concentration (MBEC) Assay was used to screen various concentrations of PAA (50 - 400 ppm) alone and in combination with the booster to determine efficacy against biofilm formed by *Pseudomonas aeruginosa*. Additional testing was performed using a CDC biofilm reactor and the Single Tube Method at various concentrations of PAA +/- booster. Sporicidal activity against *Bacillus subtilis* and *Clostridium sporogenes* was determined using a modified version of EN 13697. Endospore preps were dried on stainless steel carriers, coated with various concentrations of PAA +/- booster for 10 min followed by neutralization and recovery.

Results: In the MBEC Assay, the combination of PAA and booster achieved consistent $\geq$ 6 log kill at 100 ppm PAA whereas PAA alone required > 400 ppm. In the Single Tube Method, the combination of PAA and booster achieved > 6 log kill at $\leq$ 300 ppm PAA compared to PAA alone which required > 700 ppm. Against endospores the booster increased the sporicidal activity of PAA as much as 1 - 3 log reduction values depending on the PAA concentration.

Significance: The results indicate that the booster can increase the performance of PAA against biofilm and endospores.

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P3-52 Development of Novel Test Methods to Evaluate the Efficacy of Dry Sanitizer Products

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Introduction: Dry sanitizer products are used to control microbial pathogens present on the floors of food processing facilities and provide a barrier to reduce pathogen spread from one location to another. Antimicrobial test methods for dry products typically involve testing the product as a liquid which does not accurately reflect how the product is typically used.
Purpose: Various test methods were developed to evaluate dry sanitizer efficacy in a manner that more accurately reflects their use.
Methods: Three test methods were developed. The Dry Sanitization (DS) method involves the application of a dry product to a stainless steel carrier containing dried bacteria, followed by wetting and subsequent microbial recovery after 5 min. The Continuous Dry Sanitization (CDS) method is similar, but measures residual efficacy after multiple inoculations and wear cycles using a large boot stamp. The Cross Contamination Sanitization (CCS) method involves the application of a bacterial inoculum to a mini boot stamp which is then walked through a sanitizer and subsequent surfaces followed by microbial recovery from each surface after 5 min.
Results: Depending on the type of powder, amount, and subsequent moisture, the DS and CDS methods generated log reduction values ranging from 1.0 to 5.0 against test organisms *Klebsiella aerogenes* and *Staphylococcus aureus.* The CDS method was the most time-consuming to perform, and had a high degree of variability with low throughput. The CCS method was easier to perform, less variable, and demonstrated > 3 log reduction values even against *Listeria monocytogenes.* Furthermore, the CCS method demonstrated the ability of the sanitizer to reduce the spread of pathogens between locations.
Significance: The results indicate that it is possible to develop lab-based methods that evaluate dry sanitizer products in a more realistic manner. One or more of these methods upon standardization could be adopted industry wide and subsequently used to evaluate antimicrobial efficacy.

P3-53 Enhancing the Antimicrobial Efficacy of the Ozone Microbubble (O_3MB) in Romaine Lettuce by Altering Its Properties

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Developing Scientist Entrant

Introduction: Ozone microbubbles, which are considered an alternative treatment of chlorine for fresh produce washing, have different performances under different pH conditions. Because the effect of pH on both antimicrobial efficacy using ozone and the properties of microbubbles is still controversial.
Purpose: The purpose of this study is to evaluate the antimicrobial efficacy of romaine lettuce using ozone microbubbles under different pH solutions in association with the role of microbubbles.
Methods: The physicochemical attributes of ozone microbubbles were investigated including bubble size, size distribution, and zeta potential using a high speed camera and Zetasizer. Ozone dissolution and decomposition rate were measured by a Q45H/64 dissolved ozone meter. To evaluate the antimicrobial efficacy of ozone microbubble, 10 g of romaine lettuce was spot inoculated with *E. coli* NRRL B-3704 (10^{7-8} CFU/ml). Romaine lettuce was washed with 3ppm of ozone microbubbles at different pH (4, 6, 8) for 1 min to 20 min. Standard plate counting methods were used to analyze the survival populations. Significant differences ($P < 0.05$) in the results were determined using ANOVA.
Results: Survival populations of *E. coli* on romaine lettuce decreased by 2.8 log CFU/ml after 1 min of O_3MB treatment (3ppm) in pH 4 solution, which significantly ($P < 0.05$) showed better antimicrobial efficacy than in pH 6 and pH8 solution. Microbubbles showed different surface charges under different pH solutions, which caused unique ozone microbubble behavior to increase microbial reduction. The ozone decomposition rate in the pH 4 solution was much slower (k_d = 0.01 min^{-1}) than those in the pH 8 solution (k_d = 0.02 min^{-1}).
Significance: Our findings show how ozone microbubbles enhance reducing bacteria from romaine lettuce and help to understand the optimal pH for fresh produce washing with ozone microbubbles.

P3-54 Efficacy of Peracetic Acid and Chlorine Sanitizers to Inactivate *Cryptosporidium parvum* and *Escherichia coli* in Agricultural Water

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Developing Scientist Entrant

Introduction: Agricultural water (Ag-water) is a common source of zoonotic pathogens, specifically *E. coli* and protozoan parasites such as *Cryptosporidium parvum*. To reduce risks via irrigation, effective sanitization of Ag-water may be critical to food safety.
Purpose: Investigate the effects of aqueous peracetic acid (PAA) and chlorine on bacteria and protozoa at different treatment times and temperatures in Ag-water.
Methods: Ag-water was collected with turbidity, pH, temperature, and other key characteristics recorded. Solutions of PAA and chlorine were prepared at 1000ppm, test levels (4-6ppm, 10-12ppm, 50ppm, 100ppm, and 200ppm) were prepared via dilution from these stocks into the collected water. *C. parvum* (10^6 oocysts) and *E. coli* (EPA 6 strain cocktail of 7 logs) were assessed at 22°C and 32°C with each sanitizer, oocysts with all 5 concentrations and *E. coli* with 4-6ppm and 10-12ppm, all for 5- and 10-minute contact times in triplicate (N=360). After 5- or 10-minutes 0.1mL of Sodium metabisulfite was added to neutralize the sanitizer post treatment. *C. parvum* oocysts were exposed and infectivity quantified by HCT-8 cell culture and qPCR. *E. coli* was enumerated on MacConkey agar with 80ug/mL rifampicin. Statistical analysis was done through JMP via ANOVA.
Results: Neither sanitizer was effective against either organism at 4-6ppm. After treatment with 10-12ppm PAA and chlorine *E. coli* was significantly reduced ($p<0.05$) by 1.5 and 6 log, respectively. Exposure for 10min to a 100 or 200ppm solution of either sanitizer significantly ($p<0.05$) reduced viable oocysts by 2 and >4log. Across all experiments PAA and 32°C showed slightly greater reduction in oocysts, with neither being significantly more effective.
Significance: *E. coli* is more sensitive to PAA and chlorine compared to *C. parvum*, which required 10x the concentration of either sanitizer. Application of either sanitizer at higher concentrations in Ag-water will measurably reduce the risk of infection for *E. coli* and *C. parvum*.

P3-55 "Red Light, Yellow Light!": Evaluating the Anti-Listerial Potential of Dairy Isolate Metabolites Using a High-Throughput Chromogenic Assay

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Developing Scientist Entrant

Introduction: Controlling growth of *Listeria monocytogenes* in refrigerated food products remains a challenge for the industry. There is significant need for anti-listerial ingredients that fit other formulation constraints (e.g., clean-label, organic, sustainable).
Purpose: Identify bacterial dairy isolates that produce metabolites with anti-listerial properties.
Methods: Individual strains (n=500) from dairy facilities and nisin-producing *Lactococcus lactis* ATCC 11454 were grown in tryptic soy broth with yeast extract (TSBYE; 30°C, 20-24 hrs) in 96-well plates. Resulting fermentate (30 ml) was transferred to a 96-well plate, treated with lysozyme (10 mg/ml, 37°C, 30 min), and heat-inactivated (75°C, 30 min). *Listeria* spp. strains (n=3) were inoculated into buffered TSBYE with vital stain (2,3,5-triphenyltetrazolium chloride; BTSBYE-VS) at ~5 log CFU/ml, transferred (140 ml/well) to a 96-well plate containing the treated fermentate, and incubated (37°C, 24-120 hrs). Wells were

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observed daily for color change from yellow (no growth of *Listeria* spp.) to red (growth). Select dairy strains (n=6) demonstrating anti-listerial activity were whole-genome sequenced (WGS) and screened for bacteriocin-associated genes (BAGEL4).

Results: Thirty-two isolates (6.5%) inhibited *L. innocua* WRLP438 growth for 72 hrs. *L. monocytogenes* Ohio and California were inhibited for 120 hrs by 6 and 12 isolates, respectively. Sequencing identified strong candidates as *Bacillus licheniformis/paralicheniformis* (n=3), *B. pumilus/safensis* (n=2), or *B. velezensis/amyloliquifaciens* (n=1). BAGEL4 identified 4-6 bacteriocin constructs/isolate, including those with homology to sactipeptides, bottromycin, comX2, enterocin, halodurícin, lasso_peptides, and sonorensin. Genes with homology to amylocyclicin, closticin, geobacillin were identified in single isolates. UviB homologs were found in both strains of *B. pumilus/safensis* and *B. velezenesis/amyloliquifaciens.* The *B. pumilus/safensis* strain that inhibited all three *Listeria* strains carries two UviB homologs.

Significance: Diverse *Bacillus* isolates produce metabolites with anti-listerial properties. Genomic analysis suggests potential production of antimicrobial peptides. Future work will evaluate these strains and/or metabolites to prevent *L. monocytogenes* growth in dairy food systems.

P3-56 Genomic Analysis Identifies a Diversity of Biosynthetic Gene Clusters That Encode Antimicrobial Compounds in Rare *Salmonella* Serotypes

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Introduction: Non Typhoidal *Salmonella* (NTS) is a major cause of foodborne outbreaks, globally accounting for approximately 80 million cases of foodborne illness annually. There remains an acute need to develop approaches to reduce the presence of *Salmonella* in foods not heated prior to consumption. Natural products (encoded by biosynthetic gene clusters) are important secondary metabolites produced by bacterial species that have been reported to have antimicrobial activity.

Purpose: This study assessed the diversity of biosynthetic gene clusters in *Salmonella* and identified candidate clusters encoding antimicrobial compounds.

Methods: 2,855 *Salmonella* genomes representing 232 different serotypes contained within the *Salmonella* Food Systomics (SalFos) database were screened for the presence of biosynthetic gene clusters (BGCs) using antiSMASH (v6.1). Sequence similarity networks and gene cluster families were constructed using BiG-SCAPE (v1.1.5). BGC families were mapped against core-genome based phylogeny to infer their distribution among *Salmonella* serotypes and genetic lineages.

Results: 6,421 BGCs were identified with the great majority belonging to two structural classes, including the ribosomally synthesized and post-translationally modified peptide (RiPP) (n=3117; 48.5%) and nonribosomal peptide synthetase (NRPS) (n=2963; 46.1%) classes. The BGCs clustered into 136 gene families with cluster similarity network. Gene cluster families encoding turnerbactin, a catechol-based siderophore that is structurally similar to bacillibactin that has been reported to have antimicrobial property, were found to be conserved in this study. Different variants of these gene families were enriched in different serotypes and genetic clusters in the phylogeny. Of note, three novel gene clusters were found in rare *Salmonella* serotypes. These novel clusters contained genes encoding antimicrobial compounds including massetolide A (serotype Rubislaw), nasesеazine C (serotype Redlands), and cloacin (serotype Cotham).

Significance: This data suggests that rare *Salmonella* serotypes could be a rich source of novel gene clusters that encode compounds that have antimicrobial potential against *Salmonella* and other foodborne pathogens.

P3-57 Evaluation of Lactic Acid Bacteria Biofilms for Inhibition of Shiga-Toxin Producing *Escherichia coli* Biofilms

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❖ ***Developing Scientist Entrant***

Introduction: Shiga-toxin producing *Escherichia coli* (STEC) have caused numerous foodborne illness outbreaks and recalls. Due to their biofilm-forming capabilities, they can survive on food-contact surfaces and in food-products, despite antimicrobial treatments. Lactic acid bacteria (LAB) are known for their inhibition capabilities against STEC. However, very few studies have evaluated the efficacy of LAB biofilms to inhibit STEC biofilms.

Purpose: Evaluate LAB biofilms for inhibition against STEC biofilms.

Methods: Inhibitory capability of previously characterized LAB strains (n=65) was initially evaluated against top seven STEC serotypes (O157, O111, O26, O45, O121, O145, O103), using agar-spot-test. Biofilms of LAB, that showed excellent inhibition (zones > 10 mm), were then tested against STEC and their biofilms on polystyrene surface. Cocktails ($1x10^5$ CFU/ml) of four O157 and twelve nonO157 (2 strains per serotype) STEC were used separately to form pathogenic biofilms. LAB ($1x10^8$ CFU/ml) biofilms were formed in 96-well plates for 48 h (37/42°C; anaerobic incubation), prior to (preventive) or after (corrective) the formation of 48 h STEC biofilms (37°C). Pathogen biofilm disruption was determined by measuring absorbance (A_{595}), while STEC populations within the biofilms were determined by enumerating on appropriate media. Data were analyzed using one-way ANOVA ($P<0.05$).

Results: Of the 65 LAB strains, 15% exhibited excellent (>15 mm), 32% very-good (>10 mm), and 29% good (>5 mm) inhibition against STEC. In preventive treatments, *L. animalis* and *L. lactis* were most effective ($P<0.05$), reducing STEC populations by 4.0 and 4.3 logs, respectively, compared to the control (8.1 logs). In corrective treatments, *L. bulgaricus* and *L. casei* showed a complete reduction of STEC populations (control=8.6 logs), followed by other LAB (6.0 to 7.4 logs reduction). The LAB were more effective in reducing STEC and their biofilms as corrective treatments than preventive treatments.

Significance: Lactic acid bacteria biofilms can be used effectively to remove STEC and their biofilms on food-contact surfaces.

P3-58 Intermittent Pulsed Electric Fields for Growth Prevention of Bacteria on Leafy Greens

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❖ ***Developing Scientist Entrant***

Introduction: Microbial contamination of leafy greens continues to be an issue in the food industry, especially considering *Escherichia coli*, and prevention methods are therefore required.

Purpose: Investigate the effect of intermittent pulsed electric field (PEF) exposures on *E. coli* and *Spinacia oleracea* (spinach).

Methods: *E. coli* was fermented in electroporation cuvettes for 10 hours at ambient temperature and exposed hourly in both LB Broth and Gomori buffer. Two field strengths (0.9 kV/cm and 1.7 kV/cm) and two pulse numbers (1 and 5) were examined using the Gemini X2 electroporation system. Metabolic activity (n=8) and culture density (n=12) were measured using the XTT assay and optical density readings, respectively. Based on these results, two conditions were monitored for leaves (0.9 kV/cm x 1P, 1.7 kV/cm x 5P), exposed in Gomori buffer for the same number of times as *E. coli,* but 20 minutes apart instead. To determine leaf quality, several pre to post exposure differences were calculated, including color (L* and a*), weight, and viability by fluorescein diacetate staining.

Results: When compared to the unexposed samples, for *E. coli*, the 0.9 kV/cm x 1P condition shows a significant decrease in metabolic activity (16%, $P=0.0282$) and no significant change in culture density. The 1.7 kV/cm x 5P condition shows the largest decrease in metabolic activity (22%, $P=0.0011$) but also an increase in culture density (15%, $P<0.0001$). For spinach, quality is unaffected at 0.9 kV/cm x 1P, but is for 1.7 kV/cm x 5P, where $\Delta L^* = -3.46$ ($P=0.0019$), $\Delta a^*=.425$ ($P=0.0353$), Δweight=-20.8 mg ($P=0.0026$), and viability significantly decreases ($P<0.0001$).

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Significance: Utilization of intermittent PEFs may be used to preserve leaf quality while also slowing growth of potential contaminants by the diminishing necessary metabolic functions.

P3-59 Inactivation of *E. coli* O157:H7 on Iceberg Lettuce by Non-Thermal Plasma-Bubbling System

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❖ ***Developing Scientist Entrant***

Introduction: An integrated bubbling system is introduced to a non-thermal atmospheric plasma jet and has the potential to be an alternative for food sanitization due to the abundant presence of highly reactive oxygen species (ROS).

Purpose: This study aimed to determine the sanitation efficacy of the plasma-bubbling system in inactivating *E. coli* O157:H7 on iceberg lettuce. We also evaluated the potential of the plasma bubbling system to avoid microbial cross-contamination.

Methods: The plasma-bubbling system consisted of a plasma jet at atmospheric pressure (9 kV and 16 kHz) and was connected to a cylindrical gas injection tube. Pure oxygen (8 L/min was used as the feeding gas. Approximately 100 µl of *E. coli* O157:H7 suspension was spotted on each sliced lettuce (3×3 cm). The inoculated lettuce was air-dried inside a clean bench for 90 min. Subsequently, 10 g of the inoculated lettuce was immersed in 200 ml of distilled water, then subjected to the plasma-bubbling system for 5 min. The inactivation efficacy of plasma-bubbling treatment and the controls (100 ppm of sodium hypochlorite, distilled water, and no washing treatment) was evaluated in three independent replications and analyzed using one-way ANOVA followed by a Tukey HSD test at $P<0.05$.

Results: Washing inoculated lettuce by the plasma bubbling system reduced the bacterial load by 2.1±0.1 log CFU/g while washing with chlorine achieved 2.06±0.2 log CFU/g. Compared to both treatments, washing with distilled water resulted in a 1.2 log CFU/g reduction. Survival bacteria from the wash water of plasma bubbling treatment and chlorine solution was below the detection limit (< 1 log CFU/ml).

Significance: The plasma-bubbling system is prospective to be a food safety intervention for sanitizing fresh produce and preventing cross-contamination. The ROS generated in the system is a direct additive, so there would not be any chemical residues after the treatment.

P3-60 Outcomes of Stakeholder Meeting Discussing Outreach Efforts of Waterless, Non-Thermal Food Processing Technology USDA Coordinated Agriculture Project

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Introduction: Currently available food processing technologies may not be sufficient to address microbiological risks resulting from contamination. Researchers with the Western Center of the USDA Agriculture Research Service lead a Coordinated Agricultural Project focused on the efficacy of waterless, non-thermal food processing technologies, such as pulsed light, chlorine dioxide, and cold plasma, and developing equipment and processes for their use in food production. An integrated component of the project is to measure perceptions and acceptance of these and other food processing technologies to inform outreach efforts.

Purpose: The purpose of this study is to report the outreach-specific outcomes of a stakeholder meeting hosted by project leaders in April 2022.

Methods: Stakeholders represented academia, governmental organizations, and the food industry. Four presentations were each immediately followed by a breakout session where stakeholders were asked a series of semi-structured questions to seek their feedback and identify future directions of the work. Breakout sessions were recorded, transcribed, and coded to determine categories and themes.

Results: Related to the outreach component of the project, stakeholders highlighted the value of educating cooperative extension educators about these technologies so they can effectively educate consumers. Stakeholders acknowledged cooperative extension's role as a trusted source of information, and also challenged extension educators to take an increasingly proactive role in sharing information. Stakeholders discussed a perception that the food industry would be unlikely to utilize these technologies if consumers will not accept them. To that end, stakeholders discussed the usefulness of measuring global perceptions of food processing technologies given the international scale of our food system.

Significance: The results presented inform the remaining outreach work of the project, identify future work once the project concludes, and also to serve as a case study documenting considerations for researchers who are developing novel food processing technologies and how to effectively educate educators and consumers about them.

P3-61 Application of Novel Non-Thermal Processing Technologies in Food Protein Analysis

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Introduction: Non-thermal processing techniques such as ultrasonication and cold plasma treatment offer an alternative to conventional thermal processing as they better preserve heat-sensitive nutrients.

Purpose: This study investigates the effect of two novel non-thermal processing methods - cold plasma treatment and ultrasonication – on food protein analysis.

Methods: Phosphate buffered saline (PBS) was activated using cold plasma at a discrete frequency of 1,000 Hz for 5, 10 and 20 min, respectively. White shrimp (*Litopenaeus setiferus*) homogenates were 1:15 (g/g) extracted and egg yolk powder (Sigma, E0625) was 1:75 (g/ml) extracted using untreated and cold plasma-activated PBS. The untreated extracted proteins from two buffers were treated by a probe sonicator with a 5 s on and 25 s off pulsation for 5, 10 and 20 min, respectively, at 4 °C. Non-thermal processing-induced protein physicochemical changes, such as solubility, size distribution and zeta potential, profile, and immunoreactivity were studied using bicinchoninic acid (BCA) assay, dynamic light scattering, gel electrophoresis, and immunoblot, respectively.

Results: Cold plasma-activated buffers showed a significantly higher amount of nitrate and peroxide levels than the untreated buffers. For cold plasma-activated PBS extraction, the solubility of shrimp and egg yolk proteins was not affected ($P>0.05$) as a function of cold plasma treatment time. In addition, the immunoreactivity of two food allergens (i.e., shrimp tropomyosin and chicken serum albumin) was increased compared to untreated PBS extraction. As to ultrasonication, a 20-min treatment significantly increased the shrimp and egg yolk protein size ($P<0.05$), leading to a decrease in their stability. It also decreased shrimp tropomyosin and chicken serum albumin antigenicity by more than 40% and 30%, respectively.

Significance: Cold plasma-activated buffer extraction and ultrasonication can be applied to alter protein physicochemical properties. These techniques can be tailored to alter the antigenicity of food allergens.

P3-62 Evaluating the Efficiency of Cold Atmospheric Plasma in Inactivating *Listeria monocytogenes* in Cold-Smoked Salmon RTE

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Introduction: *Listeria monocytogenes* is a psychrotolerant foodborne pathogen, and its occurrence in RTE fish products is a significant public health concern. Cold-smoked salmon (CSS) can support the growth of *L. monocytogenes*, a major concern for smoked salmon industry. Hence, there is a need to evaluate the potential use of non-thermal processing technology to ensure safe consumption. Cold atmospheric plasma (CAP) is a non-thermal technology

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that inactivates foodborne pathogens in foods. However, extensive research is needed to optimize CAP parameters to reduce the oxidation of lipids and proteins.

Purpose: This study aimed to determine the efficiency of cold atmospheric plasma on the inactivation of *L. monocytogenes* in cold-smoked salmon.

Methods: The CSS samples were purchased from a local store. The whole sample was peeled and divided into fifteen fillets. Each fillet was inoculated with a cocktail of *L. monocytogenes* serovars inoculum. The inoculated CSS fillets were treated with cold plasma at 10 kV for 0, 3, 6, 9, and 12 min. All the samples were analyzed in triplicates, and statistical analysis was conducted at a 5 % significance level.

Results: The samples treated with CAP at 10 kV for 12 min showed the most significant ($P<0.05$) log reduction of *L. monocytogenes*, hence 1.13 (± 0.15) CFU/g was observed, followed by 1.1 (± 0.1) CFU/g at 9 min, 0.96 (± 0.11) CFU/g at 6 min and 0.53 (± 0.11) CFU/g at 3 min. Results showed that the microbial load was significantly ($P<0.05$) lower in the treated samples compared to control samples 8.9 (± 0.1) CFU/g. Hence, *L. monocytogenes* reduction efficiency depended on the treatment times ($P<0.05$).

Significance: The results show that CAP is effective in inactivating *L. monocytogenes* in CSS. Therefore, CAP has great potential to be adopted in the seafood industries to ensure safety and extend RTE food products' shelf-life.

P3-63 Isolation of Psychrotrophic Lactic Acid Bacteria to Control *Listeria monocytogenes* on Fresh-Cut Fruits during Chilled Storage

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Introduction: There has been an increase in foodborne disease outbreaks that are associated with fresh-cut fruits, especially psychrotrophic pathogens such as *Listeria monocytogenes that* thrive well at refrigeration temperatures.

Purpose: This study investigates the possibilities of using psychrotrophic lactic acid bacteria (pLAB) to inhibit *L. monocytogenes* due to its production of metabolites that are able to confer antimicrobial and antioxidant capabilities.

Methods: Isolation of inhibitory pLAB from fresh-cut fruits were performed. Isolates were screened for psychrotrophic behavior and inhibition against *L. monocytogenes*. The potential isolate was identified by whole-genome sequencing. The growth potentials at low temperature as well as *L. monocytogenes* inhibition effect on fresh-cut fruits was validated by plating out method. The spoilage potential of the isolate was evaluated.

Results: The most potential isolate (I29) was identified as *Leuconostoc gelidum*. It was able to grow well in Tryptone Soya Broth at both 4°C and 15°C, reaching up to 9 log CFU/ml after 3 days of incubation while being unable to be detected after 2 days at 37°C. On fresh-cut apple slices, I29 was able to significantly inhibit *L. monocytogenes* after 3 days of storage at 4°C (3.05±0.24 log CFU/g apple) as compared to apples without coating (4.71±0.19 log CFU/g apple) ($P<0.05$). I29 did not cause spoilage based on the measurement of pH, ˚Brix, and hardness of the apple slices at the end of storage.

Significance: This study shows the potential of using inhibitory pLAB in foods that are stored at refrigeration temperatures and allowing further investigation into enhancing its potential in edible coatings.

P3-64 Use of Infra-Red Temperature Measurements to Verify "Hot Fill and Hold" Thermal Processes for Shelf Stable Foods in Glass Containers.

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Introduction: Hot fill and hold (HFH) processes are appropriate for certain shelf stable foods. The efficacy of HFH is dependent upon the contents within a sealed container being held at prescribed temperatures and times. Process authorities provide science validated HFH processes. However, it is incumbent upon the processor to verify the thermal process parameters.

Purpose: In this study, we report on the applicability of using infrared temperature measurement (IRT) to verify food thermal process compliance.

Methods: Commercial jars (8, 16, and 32 oz) were hot filled (180 F) with distilled water (DW) or 76 Brix sugar solutions and sealed with lids fitted with an internal temperature probe. A data logger simultaneously recorded the internal (Int) liquid and external (Ext) jar surface temperatures by IRT probe at 1-minute intervals for 40 minutes. Each test combination of container size and liquid type was replicated thrice.

Results: With a DW hot filled 8 oz. jar, the initial (Int) temperature was 180.0 F, and the (Ext) container surface was 170.8 F. After a hold time of 40 minutes the Int temp was 125.4 F with an Ext temp. of 122.3 F. The same experiment but using a 76 brix solution we observed an initial Int/Ext of 180.0 and 159.0 F and 128.5/119.2 F after 40 minutes. The brix solution Int/Ext temperature differences were far greater than with the DW trials. Observations with the larger jar sizes followed a similar pattern with both solutions.

Significance: IRT may provide a rapid non-destructive approach to verify product temperatures. IRT measures surface temperature which is an indirect measurement of food within a sealed container. Nonetheless, our results have shown consistent correlations between IRT surface and product temperatures. Model equations for specific foods may be developed to accurately predict internal temperatures if IRT measurement conditions are controlled.

P3-65 Kinetic and Bio-Mechanistic Assessment of the Potential Antimicrobial Activity of UVB Treatment in Coconut Water

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Introduction: The milder Ultraviolet B radiation can be a potential antimicrobial agent, however, UVB processing to ensure food safety requires scientific attention. Therefore, the present study focuses on assessment of the antimicrobial behavior of UVB treatment in coconut water.

Purpose: To evaluate a) microbial inactivation b) comparative analysis of photoreactivation behavior of UVB and UVC treatment; and c) the mechanism of UVB induced microbial reduction.

Methods: The inactivation potential of UVB was investigated based on death kinetics of *E. coli* K12 inoculated in coconut water and exposed to the fluence level of 0 to 0.45 J/cm^2. The photoreactivation study was conducted by exposing the UVB/UVC treated sample to 1, 1.5 and 2 J/cm^2 fluence levels of UVA radiation to assess revival of inactivated microbes. Mechanism of inactivation was assessed using sub-lethally treated bacteria a) membrane damage using propidium iodide assay and TEM, b) oxidative damage using CellRox green reagent and thiol detection assay, c) metabolic activity using Resazurin assay.

Results: UVB exposure can cause up to 4.5±0.19 log inactivation in *E. coli* K12 under the dose of 0.45 J/cm^2. The UVA-mediated photoreactivation study showed there was 0.93±0.14 and 1.63±0.07 log increase in microbial load of UVB and UVC treated samples respectively. Data is indicative of a) close to 1 log inactivation was caused due to bacterial DNA damage/dimerization b) chances to microbial reactivation in food upon exposure to light is higher in UVC treated samples. Mechanistic investigations suggested that the UVB treatment significantly increases in membrane permeability, generates intracellular ROS molecules causing oxidative cellular damage and intracellular thiol oxidation. UVB mediates microbial reduction also by adversely affecting the metabolic machinery of *E. coli*.

Significance: Significant antimicrobial activity of UVB and its biomechanistic insight will lend a valuable push in evolving UVB-based technology for food safety.

P3-66 Gaseous Ozone to Improve the Microbial Safety of Spices and Nuts

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❖ ***Developing Scientist Entrant***

Introduction: An increase in reported outbreaks associated with low-moisture foods (LMFs) highlights a need to investigate non-thermal gaseous technologies to increase the safety of spices and nuts without compromising product quality.

Purpose: The objective of this study was to inactivate *Salmonella* spp. in different spices and nuts using gaseous ozone treatments and evaluate the suitability of *Enterococcus faecium* as a potential surrogate.

Methods: Four food products (basil leaves, black peppercorn, chia seeds, and walnuts) were inoculated with cocktails of *Salmonella* spp. and *E. faecium* and equilibrated to water activity (a_w) of 0.55. Two-gram samples of inoculated foods were treated in a customized chamber with ozone concentrations of 900-930 ppm at relative humidity (RH) of 70-90% for time intervals of 1-5 h, followed by mild heating at 40°C /4h. Means from 3 batches were compared with JMP Pro (significance level of 5%). Survival data were used to fit 2 primary models (Log-Linear and Weibull) to evaluate the goodness of fit.

Results: At ozone concentration of 900-930 ppm and RH of 90%, the 5 h ozone treatment resulted in maximum log CFU/g reductions of 5.0±0.4 for basil, 2.5±0.2 for black pepper, 1.5±0.2 for walnuts, and 1.1±0.1 for chia seeds. There were no significant differences between 1 and 5 h ozone treatments for chia seeds or walnuts (P<0.05). Post-treatment mild heating at 40ºC for 4 h further inactivated *Salmonella* by 1-1.5 log CFU/g for black pepper and basil. *E. faecium* was a good surrogate for *Salmonella* in basil leaves. Based on lower R^2 and RMSE values, the Weibull model provided better goodness of fit for all products.

Significance: Results of this study indicate some synergistic effects of ozone, relative humidity, and mild heating treatments to improve the microbial safety of spices and nuts during storage, though the food matrix may be a limiting factor.

P3-67 UV-C Inactivation of *Clostridium Botulinum* type B Strain in Opaque Coconut Water

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Introduction: *Clostridium botulinum* spores pose a hazard in low acid beverages (coconut water). Application of non-thermal technologies such as UV-C irradiation to inactivate endospores in foods requires an understanding of its UV sensitivities.

Purpose: To evaluate the ability of UV-C light to inactivate dormant endospores of *C. Botulinum* in coconut water.

Methods: The spores of non-proteolytic strain Type B (TJ980B) of *C. botulinum* were used for this study. The toxin serotype of *C. botulinum* spore crop was verified using the DIGoxigenin-labeled Enzyme-Linked-Immunosorbent Assay (DIG-ELISA). Optical properties of CW were measured using a double-beam spectrophotometer. UV-C irradiation was applied to stirred samples of coconut water (3mL, optical depth=0.9 mm), using a collimated beam system operating at 253.7 nm wavelength. A series of known UV doses (0 - 32 $mJ.cm^{-2}$) were delivered to spore suspension of coconut water (N=3). After treatment, the samples were heat shocked for spore's activation. Enumeration of spores was performed by serial dilution plate count method.

Results: Populations of *C. botulinum* dormant spores were reduced by ~ 3 log at the UV-C dosage of 32 $mJ.cm^{-2}$. The inactivation kinetics of spores were fitted to a log-linear model with k_{max} value of 0.24 ± 0.02 1/$mJ.cm^{-2}$) and D_{10} value of 9.41 $mJ.cm^{-2}$/log. Furthermore, ELISA results showed that there is no toxin production at UV dose of 32 $mJ.cm^{2}$.

Significance: The calculated D_{10} values will be useful to estimate the target doses required for five-log reduction of *C. botulinum* in low acidic beverages by UV-C irradiation. Further proliferation of the technology will include conducting extensive pilot studies using a continuous flow UV system.

P3-68 Microwave Pasteurization of Ready Meals

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Introduction: The pasteurization by microwaves is a process which treats quickly, prolongs the shelf life of food and minimizes the deterioration of quality compared to the conventional heating. The microwave treatment, heats directly by absorption of electromagnetic energy by the food components. This process allows an electrification of the processes.

Purpose: Show that microwave can pasteurize with better organoleptic properties.

Methods: A continuous microwave unit, was installed at CTCPA (France). The equipment consists of 24 kW microwave power - 2450 MHz - Length 5 m. An autoclave treatment versus a microwave treatment with the same pasteurization value (3000 min) will be compared. The recipe is pasta with vegetables and salmon, sweet potato puree, and green beans. 100 selected consumers will taste the two recipes and will have to evaluate the following criteria: colors, texture, taste, and overall appreciation. The tastings are organized in individual booths, in compliance with the NFV09-105 standard: the service of the cooked dishes is done according to the monadic sequential mode following a Latin square plan. The energy consumption was measured by used a EnergiMeter: Fluke 434.

Results: The results are better on all the criteria for the microwave modality, and they are significantly different (student test 5%) for the following modalities: general appreciation (7.2 vs. 6.66), color of the salmon (6.3 vs. 5.36), color of the sweet potato puree (7.79 vs. 7.66), texture of the salmon (6.05 vs. 5.3), taste of the dish (7.03 vs. 6.35). The intentions of consumption are very significantly favorable for microwave 74 vs 57 % (Chi² test significant 0.1%). Concerning the energy consumption, we go from 0.610 to 0.362 kWh/ kg of product respectively for autoclave and microwave.

Significance: The process is advantageous due to its volumetric heating principle. It can pasteurize without compromise the organoleptic properties by consuming less energy and is electrical processing.

P3-69 Minimize Post-Harvest Loss in Stored Grains by Microwaves

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Introduction: During storage, grains can be infested by insects with negative impact on its quality as well as losses. Global crop losses is between 11-14% according to previously studies.

Purpose: Show that microwave can be an efficient process for the destruction of insects.

Methods: A continuous microwave unit was installed at RISE (Sweden). The equipment consists of 54 kW microwave power - 915 MHz; product flow 3 tons of grains/hour. Grain weevils were selected for this study since it is common in infected grains and one of the more resistant species. The grains with insects (cultivated) were kept for at least 3 weeks in order to establish a collection with all differential stages, eggs, larvae, pupa and fully developed. The grains were put in sealed polyester bags and randomly mixed and for each target temperature. After, microwave bags were collected and opened to count the number of viable and dead insects, respectively. The remaining grains were transferred into the incubator. Emerging insects were counted every 2-3 days, by use of a stereo microscope, for a period of 6 weeks.

Results: We could notice a strong heat-dependent mortality after treating the insects with temperatures ranging from 50°C to 60°C. However, at higher temperatures, the mortality was higher, with heat treatment at 70°C at 8.4 seconds exposure time is sufficient to obtain up to 100% mortality (significant 5%). Concerning the germination, the values are very close to the control (92 vs 95 %). Concerning the baking properties, the following analyses were per-

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formed: raw protein ISO-12099, sedimentation value ICC-116/1, Gluten Index ICC-155, falling number ICC-107/. The values are comparable to the control (Lantmännen laboratory Sweden).

Significance: The process is advantageous due to its volumetric heating principle, efficiency and can minimize post-harvest loss in stored grains. Also is a electrical process (non-carbon emission).

P3-70 Examining Consumer Knowledge, Attitudes, and Practices of Food Irradiation to Inform Future Communications, Outreach, and Education, August-October 2022

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Introduction: Food irradiation is a safe and effective process for improving food safety yet is largely underutilized in the United States.

Purpose: We examined consumer knowledge, attitudes, and practices (KAP) of food irradiation to inform future communications, outreach, and education.

Methods: Three surveys were designed by Porter Novelli Public Services and fielded by Big Village using opt-in panel members from the Lucid platform. Survey 1 (n=1,009) fielded August 8 thru10, 2022, consisted of questions about consumer KAP. Survey 2 (n=304) fielded September 15 thru 20, 2022, asked similar questions, but was restricted to mothers with children living in the household. Survey 3 (n=1,008) fielded October 7 thru 9, 2022, focused on consumers' willingness to buy the irradiated version of select foods linked to recent outbreaks. We calculated point estimates and 95% CIs. Differences between respondents were determined using Rao-Scott's chi-square test. P-values <0.05 were considered statistically significant.

Results: When asked to choose purchasing irradiated vs. non-irradiated ground beef for an equal price, 29.8% (95% CI=26.8% to 32.8%) of respondents in survey 1 chose irradiated. This increased to 44.3% (95% CI=41.1% to 47.6%) when the price of irradiated ground beef decreased 20% and decreased to 14.1% (95% CI=11.8% to 16.4%) when the price increased by 20%. Of mothers asked to choose for their child(ren), 37.8% (95% CI=32.3% to 43.3%) chose irradiated, which increased to 43.8% (95% CI=38.1% to 49.4%) with lower price and decreased to 27.6% (95% CI=22.6% to 32.7%) with higher price. Among the 29% (95% CI=26.5% to 32.2%) of survey 3 respondents who sometimes ate hamburgers medium rare or rare, 49.5% (95% CI=43.8 to 55.3%) would buy irradiated ground beef if they wanted to eat hamburgers medium rare or rare compared with 18.2% (95% CI=13.6% to 22.8%, *P*<.0001) who would not.

Significance: These findings help characterize factors influencing consumers' decision to buy irradiated foods, which can inform future communications.

P3-71 Processing of Palm Weevil Larvae as a Novel Food Product: Innovations and Future Prospective

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Introduction: The consumption of insects as food is rapidly becoming popular. One such insect is the palm weevil. Palm weevils are large beetles, with hard shiny shells. They have been found to be a delicacy in tropical and subtropical regions. The consumption of Palm weevils as edible insects has been described to be one of the many channels to combat food insecurity. Palm weevils could serve as a nutritious supplement for expectant mothers in sub-Saharan Africa and provide an income-generating activity for local traders.

Purpose: Although palm weevils have been found to be a nutritious, cost-effective dietary supplement and environmentally friendly insects, the grubs are now being harvested with toxic substances which may endanger the health of consumers. There is therefore the need to investigate the presence and amounts of toxic substances if present. This study examines the presence of toxic substances in palm weevils harvested with chemicals.

Methods: A gas chromatographic examination of Palm Weevil Larvae Flour was conducted.

Results: Results from this study show that substances such as 5-Acetylpyrimidine, 2- Methoxyphenyl cat nitrile, 2-Chloroethyl linoleate, and Squalene were present in high quantities.

Significance: The consumption of palm weevil larvae is a plausible intervention as a nutrition supplement. However, the harvesting of palm weevils with chemicals could be injurious to health. As such, the practices of using chemicals in harvesting should be discouraged.

P3-72 Synergistic Processing Technologies Using a Combination of Olive Pomace Extract (OPE) and High-Frequency Ultrasound (HFUS) for Beverage Processing

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Introduction: The synergistic processing technologies that combine two or more antimicrobial approaches possess a significant potential to provide sustainable and safe food products.

Purpose: This study was conducted to evaluate the antibacterial activities of the aqueous extract obtained from olive pomace using ultrasound-assisted extraction (UAE) and its synergistic potential with high-frequency ultrasound (HFUS) to achieve rapid inactivation of hazardous bacteria in the juice product.

Methods: To extract the water-soluble phenolic compounds from olive pomace, dried olive pomace was suspended in distilled water (sample-to-solvent ratio: 1 g/10 ml) and bath-sonicated (100 W; 42 kHz) for 20 min, followed by membrane filtration (0.45 μm pore size). The antimicrobial synergism between the sublethal concentration of OPEs (0.1 mg GAE/ml) and HFUS (1 MHz; 1.6 W/cm^2) was evaluated using the isobologram analysis. Lastly, the plate count assay was performed to evaluate the synergistic antimicrobial activities of OPEs with HFUS against *E. coli* O157:H7 and *L. innocua* in apple juice.

Results: Using the UAE, diverse water-soluble phenolic compounds, such as gallic acid, 4-hydroxyphenylacetic acid, and hydroxytyrosol, were extracted from the olive pomace, and the total phenolic content (TPC) of the crude extract was ca. 2.65 ± 0.07 mg GAE/mL. The isobologram analysis demonstrated that the combined treatment of OPEs with HFUS exhibited strong antimicrobial synergism against *E. coli* O157:H7 (interaction index: 0.32) and *L. innocua* (interaction index: 0.58), respectively. In addition, the combined treatment enabled rapid inactivation of *E. coli* O157:H7 and *L. innocua* cells in apple juice, and both bacterial populations decreased below the detection limit (1.0 log CFU/mL) within 15 min of the treatment.

Significance: These findings provide novel information in developing sustainable technology that utilizes agricultural waste from olive oil processing, a non-toxic extraction method, and antimicrobial synergism to ensure the safety and quality of the juice products.

P3-73 Development of a Food-Grade, Bio-Based Antimicrobial Coating for Improved Microbial Safety of Fresh Produce-Contact Surfaces and Equipment

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Introduction: Cross-contamination of fresh produce with pathogens during fresh produce-handling environment is a safety and economic concern in the food industry.

Purpose: This study was conducted to develop a food-grade, bio-based antimicrobial coating that can be readily applied to the existing fresh produce-handling surfaces and reduce the microbial contamination of the surfaces.

Methods: Stainless steel (SS) was selected as a model food-contact surface and 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 SS 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

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in the simulated processing environment. To evaluate the scalability of the antimicrobial coating, a field study was conducted on a lettuce farm using the coated harvesting knife.

Results: Using the two-step coating method, the uniform coating was formed on the SS surface within 2 h, including the drying time. The coated SS surfaces showed strong antimicrobial activities against *E. coli* O157:H7 and *L. innocua*, and both bacterial populations inoculated on the coated surfaces decreased below the detection limit (1.0 log CFU/cm^2) within 10 min. Based on the sponge test, the antimicrobial coating formed on SS surfaces showed strong resistance against mechanical abrasion, without losing its antimicrobial activities after intensive cycles of dry and wet abrasions. The results of the field trial showed ca. 1.4 – 1.9 logs lower microbial loads on the coated harvesting knife than the uncoated counterpart after harvesting or trimming 100 heads of Romaine lettuce.

Significance: These findings provide practical information in developing a food-grade, biopolymer-based antimicrobial coating that can reduce the risk of microbial contamination on diverse fresh produce-handling surfaces, and thus, improve the safety and quality of fresh produce.

P3-74 Highly Sulfonated, Alginate/Polyacrylamide Hydrogel Beads for Efficient Pectinase Separation and Recovery

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Introduction: Pectinases are heterogeneous groups of enzymes widely used for clarification of fruit, vegetables juices. The use of free pectinase is inappropriate due to the difficulty of separating the free form from the reaction environment and recycling use.

Purpose: In this study we presented a scalable method for creating highly sulfonated hydrogel beads (SSAHB) for selective adsorption of pectinase from degradation broth. The obtained ion-exchange anionic beads exhibit a unique cellular structure endowing them with outstanding under compressive fatigue resistance.

Methods: The sulfonated hydrogel beads were prepared using an in situ formed network of polyacrylamide and natural polysaccharide alginate through an emulsion polymerization. Sulfonation process was obtained by simple nucleophilic substitution. The pectinase adsorption was through non-covalent interactions.

Results: The pectinase separation based on electrostatic attraction and ion exchange mechanisms which enhance pectinase adsorption capacity (250 mg.g^{-1}) within 30 minutes and recovery (98 % of adsorbed amount). Moreover, dynamic breakthrough capacity of the SSAHB could reach up to 230 mg.g^{-1}, which was almost six times higher than that of the commercial Sartorius Sartobind membrane. Furthermore, SSAHB could directly extract pectinase from degradation broth of orange juice solely driven by gravity with excellent regenerability (upon 10 cycles).

Significance: This work may provide a new avenue to design and develop next-generation high-performance separation media for recovery of free enzymes used in food industry for several times of reusing cycles significant change in its activity.

P3-75 Development of a Short Enrichment Broth for the Rapid Detection of *Bacillus* spp.

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Introduction: *Bacillus* spp. exist in various ecosystems and can cause foodborne diseases, so a procedure for enrichment above the detection limit is essential for detection.

Purpose: This study was to develop short enrichment broth for *Bacillus* spp. (SEB-B) using a response surface methodology (RSM) modified Gompertz model.

Methods: For the optimization of SEB-B, RSM and modified Gompertz model was performed. SEB-B was developed by supplementing D-(+)-Glucose (2-20 g/L) L-Proline (2-20 g/L) and magnesium sulfate (2-20 g/L) to buffered peptone water (BPW) with 2% NaCl as carbon, nitrogen, and mineral sources, respectively. To maximize the growth effect of SEB-B, pH (6.0-6.8) and temperature (27-33 °C) was optimized using conventional plate counting method. Also, to confirm the effect of the developed SEB-B, the number of *Bacillus* spp. was measured for seven hours using conventional plate counting method.

Results: SEB-B was developed by adding 15.4 g/L of glucose, 8.3 g/L of proline, and 11.8 g/L of $MgSO_4$ to BPW with 2% NaCl. To maximize the growth of *Bacillus* spp. using SEB-B, the culture conditions were optimized as 33 °C and pH 6.8. As a result of evaluating the effect of SEB-B on *Bacillus* spp., 0.82 Log CFU/ml *Bacillus* spp. was grown up to 7.83 log CFU/ml within 7 hours, while BPW with 2% NaCl enriched 0.88 log CFU/ml *Bacillus* spp. to 5.45 Log CFU/ml.

Significance: SEB-B developed in this study showed great potential as a short and rapid enrichment broth for *Bacillus* spp.

P3-76 Elimination of False-Positive Results of Thermophilic Helicase-Dependent Amplification by Combining with CRISPR/Cas12a Detection Method

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Introduction: Isothermal amplification methods have a critical problem, in which is a production of false-positive results.

Purpose: The objective of this method was to eradicate false-positive results of thermophilic helicase-dependent amplification (tHDA) by combining with clustered regularly interspaced short palindromic repeats (CRISPR)/Cas12a method.

Methods: tHDA was used for the amplification of *stx2* in *E. coli* O157:H7. Lateral flow assay (LFA) was employed to show the production of false-positive result in tHDA. CRISPR/Cas12a was employed for the eradication of false-positive produced by tHDA. Specificity of tHDA-based CRISPR/Cas12a was performed with 3 target strains and 12 non-target strains. Sensitivity was analyzed in both pure culture and food sample.

Results: The results showed that tHDA produced false-positive results on LFA, whereas no false-positive results were produced when combined with CRISPR/Cas12a. Specificity test resulted that only the target strains showed fluorescens. In addition, tHDA-based CRISPR/Cas12a detected up to 10^3 CFU/mL and 10^4 CFU/mL of *E. coli* O157:H7 in pure culture and mixed salad, respectively.

Significance: This study provided great potential in eliminating the false-positive results, thereby provided significance in the food industry.

P3-77 Loop-Mediated Isothermal Amplification-CRISPR/Cas12a Based on Lateral Flow Biosensor for Sensitive and Visualized Detection of *Salmonella*

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Introduction: The clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated Protein 12a (Cas12a) is attracting attention as a biosensing technology because it can generate fluorescence or colorimetric readouts.

Purpose: This study was to develop a loop-mediated isothermal amplification (LAMP)-CRISPR/Cas12a based on a lateral flow biosensor (LFB) that can detect *Salmonella* simply and inexpensively through visual analysis.

Methods: The LAMP was performed at 65°C for 60 min, and for visual detection of LAMP amplicons by lateral flow biosensor, the optimal concentration of ssDNA reporter was examined from 0 to 50 nM and the reaction time of Cas12a-mediated trans-cleavage was examined from 0 to 20 min. A group containing a reactive component and a group lacking a specific component were compared to test the Cas12a-mediated trans-cleavage activity of Cas12a. The sensitivity and specificity of LAMP-CRISPR/Cas12- based LFB were evaluated in pure culture and foods.

Results: The ssDNA probe captured by streptavidin was fixed to the T line and had a red color, but the ssDNA cleaved by CRISPR/Cas12a was not immobilized on the T line. We chose the reaction conditions of 5 min with 5 nM ssDNA reporter as the optimal Cas12a-mediated trans-cleavage conditions.

The ssDNA probe captured by streptavidin was fixed to the T line and had a red color, but the ssDNA cleaved by CRISPR/Cas12a was not immobilized on the T line. In addition, the LAMP-CRISPR/Cas12a based on lateral flow biosensor using filtration successfully detected 10^1 CFU/g of *Salmonella* in 25 g of red onion, melon, and salami without pre-microbial enrichment culture. The filtration, DNA extraction, LAMP, Cas12a cleavage, and LFB lasted for 5, 20, 60, 5, and 5 min, respectively.

Significance: This developed method can be generalized for a variety of pathogens and be a promising biosensing technology with better field applicability.

P3-78 Filtration-Based RPA-CRISPR/Cas12a System for the Rapid, Sensitive and Visualized Detection of *Salmonella*

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Introduction: In order to control *Salmonella* outbreak, which causes serious foodborne diseases in humans worldwide, rapid and accurate diagnosis is essential, and the development of point-of-care testing (POCT) applicable molecular diagnostic methods is important.

Purpose: This study developed a DNA endonuclease-targeted CRISPR trans reporter (DETECTR) technology in combination with recombinase polymerase amplification (RPA) and clustered regular interspaced short palindromic repeats (CRISPR)/Cas12a system to develop a POCT-capable molecular diagnostic method that can rapidly and sensitively detect *Salmonella*.

Methods: The RPA primers were designed to target the *InvA* gene. Additionally, for visual detection of RPA amplicon, the crRNA for the CRISPR detection system is specifically designed for the amplified region of the PAM site. Then, we optimized the primer concentration, amplification temperature, magnesium acetate (MgOAc) concentration, and amplification time of the RPA system to obtain the maximum fluorescence intensity of the DETECTR technology. Specificity tests were performed to validate the DETECTR technology, and sensitivity tests were performed at 10^0–10^6 CFU/mL of *Salmonella*. A filtration-based bacterial enrichment was performed without a culture-based enrichment process.

Results: The developed DETECTR technology amplified only *Salmonella* in the specificity analysis and detected up to 10^2 CFU/mL of *Salmonella* in the sensitivity analysis. The pretreatment method, including the filtered enrichment procedure, increased the sensitivity 10 to 100-fold and was performed within 5 min. RPA was performed at 37 °C within 20 minutes, and Cas12a cleavage was performed at 37 °C within 5 minutes, which could be confirmed visually.

Significance: The method developed based on the designed primers increases the applicability of POCT without expensive equipment and can be used to detect *Salmonella* rapidly and sensitively.

P3-79 CRISPR/Cas9 Mediated Genome Editing of T4 Bacteriophage for High-Throughput Antimicrobial Susceptibility Testing

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❖ ***Developing Scientist Entrant***

Introduction: The accurate and effective determination of antimicrobial resistance is essential to limit the spread of infection diseases and ensure food safety.

Purpose: Herein, a simple, inexpensive, and high-throughput phage-based colorimetric sensing strategy was developed for antimicrobial susceptibility testing.

Methods: Taking advantage of the CRISPR/Cas9 system, the genome of T4 phage was modularly engineered to carry LacZ, a marker gene encoding β-galactosidase. $T4_{LacZ}$ phages were identified by blue/white selection and then used to construct a sensing application. In this strategy, the addition of $T4_{LacZ}$ phage to the bacterial solution can infect target bacteria to overexpress β-galactosidase, which triggered an enzymatic reaction in the presence of a colorimetric substrate, resulting in a color change. This sensing strategy offers a visual way to monitor bacterial growth in the presence of antibiotics, enabling the determination of antimicrobial susceptibility.

Results: As a proof-of-concept, the proposed sensing strategy was applied to identify multidrug-resistant *Escherichia coli*. The *E.coli* were cultured in the liquid media contains different antibiotic. After 3-hour preincubation, the $T4_{LacZ}$ phages and colorimetric substrate were added in the bacterial solution. The enzymatic reaction was triggered during the phage infection. Therefore, the antimicrobial resistance of *E. coli* can be confirmed by analyzing the colorimetric response of the sensing system. Under the optimized conditions, this antimicrobial susceptibility testing method successfully identified 11 different multidrug-resistant *E. coli* with 100% specificity.

Significance: Compared with conventional disk diffusion susceptibility tests, the phage-based sensing strategy can shorten the detection time by at least half without losing the detection sensitivity, providing an alternative method for antimicrobial susceptibility testing in the food supply chain.

P3-80 Evaluation and Application of a Next-Generation Sequencing Panel for Detection and Identification of Multiple Pathogens of Fermented Foods in One Reaction

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Introduction: It is important to detect and identify foodborne pathogens in foods for food safety. NGS panel was suggested as an alternative method for rapid detection and accurate identification of multiple pathogens in one reaction.

Purpose: This study aims to develop and evaluate the NGS panel method for detection and identification of multiple outbreak origin pathogens of fermented foods in one reaction.

Methods: To identify target pathogen-specific genes, pan-genome analysis was performed using panX and ANVIO programs with their complete genome sequences in public databases. Among those genes, 13 specific virulence factor genes were selected from five types of pathogenic *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* Typhimurium, respectively. Based on those genes, new primer sets for NGS panel, targeting one to five gene(s) per target pathogen, were designed using Primer3 program. Primer set specificity was validated using conventional PCR methods. To evaluate the NGS panel, this method was conducted with six artificially contaminated fermented food samples using those primer sets. After mapping to 13 different target genes of seven target pathogens, all qualified NGS panel sequence reads were collected and used for identification of the pathogens.

Results: NGS panel analysis showed that average of the mapped sequence reads targeting pathogen-specific genes was obtained: 161,081, 28,929, 1,765, and 237 at 10^8, 10^7, 10^6, and 10^5 CFU per target pathogen, respectively, indicating that the average of mapped sequence reads and CFU per target pathogen was proportional. All target genes were multi-detected in one reaction at 10^8-10^5 CFU of target strains, but a few false-positive results were shown at 10^6-10^5 CFU. To validate this NGS panel, three sets of qPCR analyses were independently performed with the same samples, showing the similar specificity and selectivity.

Significance: This study suggests the efficiency and usability of NGS panel for rapid determination of origin strain in various foodborne outbreaks in one reaction.

P3-81 Microbial and Chemical Qualities and Bacterial Community in Mustard Pickle Products, a Traditional Fermented Vegetable in Taiwan, Determined Using High-Throughput Sequencing

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Introduction: Mustard pickle (Brassica juncea) is a fermented vegetable with a sour taste and is the most common traditional fermented food in Taiwan.

Purpose: Full-length sequencing of the bacterial 16S rRNA gene in the samples was performed using PacBio SMRT to analyze the bacterial community in mustard pickle products.

Methods: Microbial and chemical qualities and microbiomes of 14 mustard pickle products coded sequentially in traditional Taiwanese markets.

Results: The results showed that no coliform, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Listeria monocytogenes* were detected in any of the samples. Analysis of the chemical quality showed that the sulfite content of all samples (100%, 14/14) exceeded 30 ppm, which is the food additive limit in Taiwan. Furthermore, samples A, C, E, and J were analyzed using high-throughput sequencing (HTS). The results of HTS showed that mustard pickle product samples from different sources contained different microbiomes. The dominant bacterial family was Lactobacillaceae in all samples except for in sample A. In contrast, the microbiome of sample A mainly consisted of Morganellaceae and Vibrionaceae, which may have resulted from environmental contamination during storage and sales.

Significance: The sulfite contents from bleach in all commercially available mustard pickle products exceeded the allowable limit of food additives (30 ppm), with a failure rate of 100%. Although the samples contained no food pathogens, the high-throughput sequencing results revealed potential environmental contamination in samples.

P3-82 Detection of 1–5 CFUs of *Salmonella* in 750 g Confectionery Samples after 18 Hours with Hygiena® Real-Time PCR Assay and Different DNA Isolation Options

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Introduction: A fast and easy diagnostic tool for detection of *Salmonella* in food is essential to minimize the impact on humans and the food market due to *Salmonella* outbreaks. Being able to react more quickly to salmonellosis outbreaks than ISO 6579 examination allows, it is common to use alternative PCR methods and more and more pooling of samples in ever larger batches. Even so, it must be guaranteed that the presence and absence of *Salmonella* can still be analyzed for these large sample sizes.

Purpose: The objective of this study was to determine the sensitivity of a *Salmonella* real-time PCR Kit with 750 g and 250 g samples of seven chocolate production matrices with a 16-hour enrichment in 0.1 and 3.5% milk.

Methods: For each matrix-, 750 g, 250 g samples or swabs were inoculated with dry- and heat-stressed *Salmonella* strains with a concentration of 1-5 CFU/sample. After a 16-hour enrichment in 0.1 and 3.5% UHT milk (additional swabs in BPW), the sensitivity of the Hygiena® foodproof® *Salmonella* Detection LyoKit was evaluated. For variable applications, three different DNA isolation methods were tested for automatic and manual use in combination with the PCR. For result comparison, the ISO 6579 analysis was conducted in parallel.

Results: The study was able to confirm that the Hygiena® alternative method for *Salmonella* detection in 0.1 and 3.5% milk enrichments for 16 hours does not lead to a loss of sensitivity, even with a sample size of 750 g chocolate production matrices. The sensitivity is independent of the DNA isolation method. Overall, the results of the alternative method showed 99.5% agreement with the results of the reference method.

Significance: 1-5 CFUs of *Salmonella* can be detected in challenged 750 g confectionary matrix samples within 18 hours.

P3-83 Development and Internal Validation of the Hygiena® foodproof® *Salmonella* Plus *Cronobacter* Detection Lyokit

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Introduction: The recall of powdered infant formula (PIF) in 2022 due to *Cronobacter* and *Salmonella* infections in newborns once again demonstrates the importance of testing human food products, especially PIF, and environmental samples for these two pathogens.

Purpose: The aim of this study was to develop a rapid real-time PCR assay for the simultaneous detection of *Salmonella* and *Cronobacter* in combination with foodproof® StarPrep® One, foodproof Magnetic Preparation Kit I and BAX® System lysis reagents as different options for DNA extraction.

Methods: Sensitivity studies were conducted using a dilution series of purified DNA and enrichment cultures spiked with *Salmonella* or *Cronobacter*. PIF was incubated in BPW (1:10 dilution) at 37 °C for 18 hours. Following enrichment, samples were inoculated with different concentrations of *Salmonella* Enteritidis and *Cronobacter sakazakii*. DNA isolation was performed manually (single tube and 8-strip formats) and automatically using magnetic bead technology, followed by real-time PCR analysis. Additionally, 375 g of PIF samples were evaluated by using the BAX System DNA extraction reagents and the BAX System Q7 instrument for PCR. Specificity testing included 48 *Salmonella* strains, 46 *Cronobacter* strains and 47 non-target strains.

Results: Sensitivity studies demonstrated that both target organisms could be detected at a minimal concentration of 5 genome equivalents of purified DNA and at minimal concentrations of 10^2 – 10^3 CFU/mL enrichment culture. The real-time PCR kit could also be used with BAX lysis reagents on the BAX Q7 System. The specific studies resulted in 100% inclusivity for the 48 *Salmonella* and 46 *Cronobacter* target strains with 100% exclusivity of 47 non-target strains.

Significance: Providing the dairy and infant formula industry with a rapid, reliable and easy-to-use PCR method for the detection of *Salmonella* and *Cronobacter.* By combining these two parameters in one single real-time PCR reaction, testing time and costs are significantly reduced.

P3-84 Detection of Shiga Toxin-Producing *Escherichia coli* (STEC) on Micro Tally™ Swabs and in 375 g Samples of Ground Beef, Beef Trim and Leafy Greens by Real-Time PCR

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Introduction: STEC are known to cause diarrhea, hemorrhagic colitis and the potentially fatal hemolytic uremic syndrome. The main sources of STEC infections are raw beef, raw or inadequately pasteurized milk, sprouts and vegetables. Hygiena's foodproof® STEC Screening LyoKit detects the Shiga toxin genes *stx1* and *stx2* as well as the *eae* gene which codes for the adherence factor intimin in three different detection channels within one real-time PCR reaction.

Purpose: The aim of this study was to investigate the applicability of the foodproof STEC Screening LyoKit in combination with the BAX® System MP enrichment broth, the BAX System DNA extraction procedure and the BAX System Q7 instrument.

Methods: Eight artificial contaminated (six low-level and two high-level) and three uninoculated samples per matrix were tested for each of the seven major STEC serogroups (O157, O26, O45, O103, O111, O121, O145). Each MicroTally swab was diluted in 400 mL BAX MP Media and 375 g samples of ground beef, beef trim and leafy greens were diluted in BAX System MP Media (1:4). All test portions were incubated at 42 °C. DNA was isolated at different time points with the BAX System lysis procedure, followed by real-time PCR analysis on the BAX System Q7 instrument.

Results: For each matrix and timepoint, 88 datapoints were generated. On MicroTally swabs and in 375 g samples of ground beef, beef trim and leafy greens, all STEC strains used for spiking could be detected with the workflow tested.

Poster

Significance: These results provide the food industry with a reliable method for the rapid detection of STEC in the most relevant matrices.

P3-85 Development of Hygiena® Real-Time PCR Assay for the Detection and Quantification of Cheese-Spoiling *Clostridia* in Raw Milk

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Introduction: Some clostridia are of great concern for the dairy industry because they are the causative agent of gas defects such as "late blowing" of hard and semi-hard cheeses. *C. tyrobutyricum* is considered the primary cause, but *C. butyricum*, *C. beijerinckii* and *C. sporogenes* could also contribute to these defects. Traditionally, clostridial spore numbers were estimated by most-probable number (MPN) assays based on gas production in anaerobically incubated samples. These classical methods have some drawbacks because they are laborious, very time-consuming and the results depend on the enrichment conditions used. Another important drawback is the low selectivity because facultative anaerobic spore formers may also be detected.
Purpose: The foodproof® Spoilage Clostridia Quantification LyoKit was developed as a specific alternative method that enables a sensitive quantification of the four cheese-spoiling *Clostridium* species in a few hours.
Methods: Specificity was evaluated by testing a panel with more than 60 isolates total, with 25 isolates of the 4 target species. The sensitivity was determined using purified DNA for the PCR assay itself as well as with milk samples spiked with spores for the complete workflow. DNA isolation was conducted with a dedicated protocol based on the **food**proof StarPrep® Two Kit using 8 ml of milk.
Results: The specificity of the kit (100%) was confirmed from the inclusivity and exclusivity panel. A LoD (95%) of 2 to 5 DNA copies/reaction was determined. Detection down to ~100 spores/liter of raw milk was possible with the developed method in 4 to 5 hours.
Significance: Providing the cheese industry with an easy-to-use PCR method for spoilage clostridia reduces the time to results from several days to a few hours and the higher selectivity can drastically improve the production of hard and semi-hard cheeses.

P3-86 Development and Validation of Hygiena® Real-Time PCR Assay for the Detection and Identification of *Aspergillus* Species in Cannabis and Hemp

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Introduction: *Aspergillus* is a common mold that can produce mycotoxins and cause serious health problems like aspergillosis, especially in immunocompromised individuals or people suffering from lung disease. In the USA, several cases of illness and death following the consumption of medicinal and recreational cannabis have led to the regulation of *Aspergillus* in these products.
Purpose: The aim was to provide a real-time PCR assay that detects the species *Aspergillus flavus, A. terreus*, *A. niger* and *A. fumigatus* in a single reaction and according to AOAC SMPR® requirements for the detection of *Aspergillus* in cannabis.
Methods: After sample enrichment for 48±2 hours at 37±1°C in buffered peptone water with chloramphenicol (100 mg/L), DNA extraction was performed with either the foodproof® StarPrep® Two Kit or BAX® System lysis reagents for Yeast and Mold. Lysates were analyzed with the newly developed foodproof *Aspergillus* Detection LyoKit for the simultaneous detection and identification of the species *A. flavus, A. terreus*, *A. niger* and *A. fumigatus*. An internal amplification control prevented misinterpretation of negative results due to inhibition.
Results: The PCR kit was successfully tested with cannabis and hemp matrices. The specificity studies confirmed 100% specificity for 41 target and 81 non-target strains. In the sensitivity study, the use of the PCR kit in combination with extraction and enrichment methods resulted in a relative detection limit of 1-10 CFUs per sample for all matrix categories and protocols tested. All PCR results were comparable to the reference culture methods for the detection of yeast and molds.
Significance: The method is currently being validated according to AOAC SMPR requirements for the detection of *Aspergillus* in cannabis. The kit includes all the needed real-time PCR reagents to detect and identify the four pathogenic *Aspergillus* species along with a control template.

P3-87 Validation of Polyskope Media for the Detection of *Listeria monocytogenes* in Environmental Swab Samples Utilizing Three PCR Methods

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Introduction: *Listeria monocytogenes* is a pathogen of concern for many food processors. Environmental swabs are used to detect *Listeria* spp. as an indicator for the potential presence of *L. monocytogenes*. If *Listeria* spp. is present corrective actions are taken to mitigate potential *L. monocytogenes* presence.
Purpose: To validate PolySkope One Environmental Multiplex (POEM) media for detection of *L. monocytogenes* in environmental swab samples using different PCR platforms. The PolySkope One multiplex PCR, BAX® Real Time PCR and GENE-UP® Real Time PCR were used in this study.
Methods: Environmental swab StickSponge™ (Hygiena) samples (n=60) were taken at various locations of a meat processing plant to collect background flora. Samples were inoculated with *Listeria monocytogenes* (2 CFU/sample for 40 samples (low), 10 CFU/sample for 10 samples (high) and 10 samples were not inoculated). One-hundred milliliters of POEM was added to each sample. The swabs were then homogenized for 30 seconds and incubated at 37±1ºC for 23±1 hours. Lysates were created for each platform and analyzed according to manufacturer's instructions. All samples were subjected to culture confirmation according to USDA FSIS MLG 8.13.
Results*:* All control-samples were negative for culture confirmation. Nine-out-of-10 (90%) high-inoculation-samples and 29-out-of-40 (72.5%) low-inoculation-samples were positive for culture confirmation. High-inoculation-samples were all positive in all PCR platforms. The PolySkope method had 1 positive for the control-samples, but all control-samples were negative for the BAX and GENE-UP methods. PolySkope, BAX and GENE-UP had 28 (70%), 26 (65%) and 24 (60%, respectively) positives out of 40 samples for the low-inoculation-samples. All methods were not statistically different than culture confirmation according to Probability of Detection.
Significance: The PolySkope One Environmental Multiplex (POEM) media was validated for the detection of *L. monocytogenes* using three different PCR platforms on environmental monitoring samples.

P3-88 Specific and Accurate Detection of *E. coli* O157:H7 in Salads by Immunomagnetic Separation and Pmaxx-qPCR

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Introduction: Sensitive and rapid detection of *E. coli* O157:H7 is important in the food safety industry because *E. coli* O157:H7 causes foodborne diseases worldwide and the symptoms are dangerous.
Purpose: The purpose of this study was to use immunomagnetic separation (IMS)-propidium monoazide (PMAxx)-qPCR to detect trace amounts of *E. coli* O157:H7 in a salad.
Methods: IMS was performed by treating beads in 1 ml bacterial solution containing 10^5 CFU/ml of *E. coli* O157:H7 using optimized IMS conditions. Then, the resuspended solution was treated with 25 µM PMAxx to extract DNA, and qPCR was performed. Specificity was evaluated by testing with *E. coli* O157:H7 and non-*E. coli* O157:H7 strains, and sensitivity was evaluated in *E. coli* O157:H7 at 10^0 to 10^6 CFU/ml.

Results: PMAxx treatment effectively inhibited the proliferation of dead cells. Through a specificity test, it was confirmed that *E. coli* O157:H7 was specifically amplified. In the sensitivity test, IMS-PMAxx-qPCR detected 10^1 CFU/ml of *E. coli* O157:H7. 10^2 CFU/g of *E. coli* O157:H7 was detected in the salad using IMS-PMAxx-qPCR.

Significance: Because the IMS-PMAxx-qPCR method can effectively inhibit the amplification of dead cells, it can reduce overestimation due to dead cells, making it promising for use in the food safety industry.

P3-89 Food Safety of Hydroponic Fresh Produce: An Evidence Synthesis

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❖ ***Developing Scientist Entrant***

Introduction: A literature review is warranted to identify and retrieve evidence relevant to the food safety of hydroponic crops and appraise and synthesize the data to explore potential avenues of research, build food safety guidelines, and inform industry practices and regulatory policies.

Purpose: To review and synthesize published evidence on hydroponic food safety research and provide directions for further studies that will support the development of food safety guidelines.

Methods: A systematic review approach was used to comprehensively search and review relevant papers published between 1984 and 2022. PubMed and CAB databases were used to screen and extract relevant articles using the terms 'Pathogens,' 'Produce,' and 'Hydroponic.

Results: From 671 deduplicated records reviewed by title and abstract, 582 were excluded. Among the remaining 89 articles, relevant topics included interventions in hydroponic farming (n=31), food safety risk factors (n=36), and prevalence (n=11) or occurrence (n=24) of human pathogens in hydroponic crops. The records were categorized into pathogen survival (n=68) and pathogen internalization (n=29) studies. Most of the studies (n=86) were published after 2000. While most studies (74%) did not report which production system they studied, 17% were performed in NFT, and 9% others (e.g., Aquaponics). Most studies investigated leafy greens (83.5%), followed by seeded vegetables (15%) and 1.5% melon and berries. Most articles investigated food-borne bacteria (84%). Viruses, parasites, spoilage yeast, and molds accounted for the other 16% of the articles. The primary food safety risk factor investigated was water and water management (n=31). Interventions for pathogen control included the use of sodium hypochlorite (13%), UV-light (13%), and other (e.g., pH, acids, electrolyzed water, etc.; n=74%).

Significance: This study provides insight into the extent and themes of existing evidence in hydroponic food safety. Further data extraction and quality assessment are needed to appraise the literature and identify the data gaps fully.

P3-90 Eliminating *Salmonella* Typhimurium from Lettuce Grown in Nutrient Film Technique (NFT) Hydroponic System for Improved Food Safety and Nutrition

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Introduction: Hydroponic farming systems play an increasingly vital role in the sustainable production of nutrient-rich food. Although there are food safety risks in hydroponic production, validated sanitation food safety protocols are lacking. The effectiveness of sanitizers in eliminating human pathogens from hydroponic surfaces, and their effect on crop quality and nutrient composition are unknown.

Purpose: This study aimed to evaluate the effectiveness of SaniDate (previously found sanitizer efficient against human pathogens) and pH-manipulated nutrient solution in eliminating the human pathogen Salmonella Typhimurium while conserving lettuce quality and nutritional attributes.

Methods: Using a randomized completed block design, *Salmonella* Typhimurium (LT2JSG626) was inoculated (8 log CFU/ml) into NFT system with lettuce and treated with SaniDate12.0 (200ppm) or reduced pH (pH 4, 5). Crop health (yield, color) and nutrient concentrations (Provitamin A, Vitamin C, lutein) were examined by weighing and using HPLC-DAD, respectively.

Results: SaniDate treatment significantly reduced lettuce yield (P=0.03). Compared with the non-treated control (193.2±15.2g/plant), the yield of treated crops was reduced by 57.2%. The weight did not significantly differ for pH treatments (P= 0.11). Compared to the non-treated control, bacterial reduction was observed across all treatment groups. SaniDate 12.0 was most effective in reducing the pathogen concentration on lettuce roots (P<0.004). The lettuce quality measured by percentage discoloration was 17.6%, 6.2%, and 10.3% for pH 4, pH 5, and SaniDate 12.0, respectively. Beta-carotene measures were in the range of 18.4 mg/100g of dry lettuce, consistent with expected levels for the lettuce type. Overall, pH 4 manipulated nutrient solution had an overall most efficient bacteria reduction (3.48±0.59 log CFU/g on root and 3.48±0.44 CFU/g on rockwool), with the lowest quality trade-offs.

Significance: The study provided information for producers and stakeholders to use in developing standards and protocols for hydroponic system sanitation and crop contamination prevention with human pathogens.

P3-91 Surface Dielectric Barrier Discharge Plasma for in-Package Inactivation of *E. coli* O157:H7 Biofilms on Baby Spinach Leaves

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❖ ***Developing Scientist Entrant***

Introduction: Biofilms are resistant to conventional inactivation methods. Cold plasma, the 4th state of matter, has shown promise in sanitation of produce. In our previous study, paper-based surface dielectric barrier discharge (SDBD) electrodes were used for in-package sanitation of attached bacteria on produce. However, there is a need to improve the longevity of the electrodes and test inactivation on resistant biofilms.

Purpose: This study aimed at developing new Kapton-based SDBD electrodes with a longer lifespan and at determining their inactivation efficacy on *E. coli* O157:H7 adherent bacteria and 24 hour grown biofilms on baby spinach.

Methods: The Kapton-based electrodes were fabricated using sheets of aluminum and polyimide thermoplastic (Kapton) and were characterized by ozone generation and lifespan. Spinach leaves were inoculated with *E. coli* O157:H7 as adherent cells or grown into biofilms. Adherent cells were dried at room temperature for 90 minutes and biofilms were grown for 24 hours at 4 °C before treatment. Kapton-based SDBD electrodes were used for the treatment of leaves (at 1.6 kHz and 5 kV peak-to-peak voltage) for 2, 5, 10, or 15 min. Three independent replicates were performed, and the data were analyzed using the student's t-test for equal variances (P<0.05).

Results: Kapton-based SDBD electrodes generated 7 times more ozone than the previous paper-based electrodes. Plasma treatment reduced adherent bacteria by 2.0±0.02 and >4.6±0.3 log CFU (initial 4.62±0.27 log CFU/leaf) at 2 min and 15 min treatments, respectively. The reductions in biofilms were lower, which were0.9±0.3 to 2.9±0.7 log CFU/leaf (initial 4.2±0.4 log CFU/leaf) for 2 min and 15 min, respectively. In general, an increasing trend in *E. coli* O157:H7 inactivation was observed as a function of treatment time. Reductions from all treatments were different than each other (P<0.05) except for 10 and 15 min.

Significance: New Kapton-based SDBD electrodes have the potential as an innovative in-package sanitation method for fresh produce against bacteria and biofilms.

Poster

P3-92 Effects of High Voltage Atmospheric Cold Plasma to Inactivate *Aspergillus flavus* on Raw Peanut Kernels

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Developing Scientist Entrant

Introduction: Peanuts are highly susceptible to be contaminated with *Aspergillus flavus* in the field or during storage. However, *Aspergillus flavus* spores are resistant to several food decontamination methods and can also produce heat stable aflatoxins, which are hazardous to people and animals.

Purpose: This study investigated the effects of High Voltage Atmospheric Cold Plasma (HVACP) treatments to inactivate *Aspergillus flavus* spores on peanuts and evaluated the peanut quality changes after HVACP treatments.

Methods: 50ul (~6.8 log CFU/g) of *Aspergillus flavus* spore suspension was spot-inoculated on 10 g of peanut kernels before High Voltage Atmospheric Cold Plasma treatment, then recovered with Maximum Recovery Diluent (MRD) and plated with Potato Dextrose Agar (PDA). Plates were incubated at room temperature for 2 to 3 days before mold spores counting. Different treatment time (2, 5, 10 min), air relative humidity (5%, 40%, 80%RH), and post-treatment storage (0, 4, 24h) were applied as parameters during 90KV direct exposure HVACP treatments (all treatments were conducted in triplicates). The HVACP treatment condition which illustrated the highest microbial reduction was applied for quality assessments. Color (L*, a*, b*), moisture content, peroxide value (PV), and textural parameters of peanut kernels were analyzed before and after HVACP treatment.

Results: HVACP working with 80%RH Air and 24h-post storage treatment reduced ~1.84 log (P<0.05) and ~3.51 log (P<0.05) of *Aspergillus flavus* from peanut kernels after 2 min and 10 min treatments separately. The quality parameters of color, moisture content, peroxide value, fracture force, and hardness showed no significant difference (P>0.05) between treated and untreated peanuts.

Significance: High Voltage Atmospheric Cold Plasma is a non-thermal, low energy, and economical technology, which has been proved as an effective alternative to current techniques to assure both the safety and quality of peanuts.

P3-93 Inactivation of *Aspergillus flavus* on Green Coffee Beans by Treatments with Organic Acid Vapor

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Developing Scientist Entrant

Introduction: Coffee beans are frequently spoiled by molds including *Aspergillus* flavus which produces aflatoxin, a human carcinogen, during post-harvest.

Purpose: The objective of this study was to inactivate *A. flavus* on green coffee beans using organic acid vapor.

Methods: Green coffee beans inoculated with *A. flavus* spores (5.2 to 5.9 log CFU/5 beans) were treated with vapor (100 mg/L) generated from acetic acid, formic acid, or propionic acid at 25 or 50°C (RH 43 or 100 %) for up to 6 h. After the treatment, the number of *A. flavus* and color and pH of the beans were measured. In addition, the beans treated with organic acid vapor were stored at 12 or 25°C (RH 100 %) for up to 14 days and mold growth was observed with naked eyes during the storage. The results were analyzed by the general linear model procedure of SAS software.

Results: The numbers of *A. flavus* on the beans exposed to acetic acid or propionic acid vapor for 6 h decreased by 1.2 to 3.1 or 0.5 to 3.3 log CFU/5 beans, respectively. When green coffee beans were treated with formic acid vapor for 6 h, the microbial population decreased to below the detection limit (1.7 log CFU/5 beans), regardless of temperature and RH. Formic acid treatment did not change the color (Hunter's *L*, *a*, and *b* values) of the beans; however, pH of the beans significantly decreased after the treatment. Mold did not grow on the beans treated with formic acid vapor during the storage, whereas molds were observed from untreated beans and beans treated with acetic acid or propionic acid after 5 days of storage.

Significance: Treatment of organic acid vapor significantly reduced populations of *A. flavus* on the beans and high temperature or RH resulted in a synergistic effect of organic acid vapor on mold inactivation.

P3-94 Combined Disinfection Effects on Mung Bean Seeds to Control *L. monocytogenes* in Mung Bean Sprouts

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Introduction: Multiple foodborne outbreaks with the consumption of mung bean sprouts have been reported worldwide.

Purpose: This study investigated the combined effects of mild heat, slightly acidic electrolyzed water (SAEW), UVC LED, and bacteriophage to control foodborne pathogens in mung bean seeds and sprouts.

Methods: Mung bean seeds are dipped in an inoculum solution of *L. monocytogenes* (isolated from smoked salmon and enoki mushroom) for 2 min. The contaminated seeds are treated with mild heat at 40, 50, and 60°C for 1 and 3 min, 30 ppm SAEW for 5 min, UVC LED for 5 min, and 2% bacteriophage (Phage guard listex, Micreos). The 5g of treated seeds were placed in a sprouting machine and kept in a humidity chamber (25°C , 85%) for 4 days. The effects of various treatments on germination rates and reduction of foodborne pathogens in seeds and mung bean sprouts were evaluated.

Results: The germination rates were not significantly affected by each treatment (94.2%~98.8%) compared to the control (98.5%). The highest reduction effect of *L. monocytogenes* was observed in mung bean seeds and sprouts treated with 60°C for 3 min among mild heat treatments. When mung bean seeds are treated with SAEW, UVC LED, and bacteriophage, the highest reduction was observed with 30 ppm SAEW (38.96%), followed by UVC LED (13.25%) and bacteriophage (6.49%). In the treated sprouts, the highest reduction of *L. monocytogenes* was observed with the treatment of bacteriophage (37.53%), followed by mild heat treatment at 60°C for 3 min (36.69%), compared to untreated sprouts.

Significance: The application of optimal combined treatment will be important for reducing microbial contamination on mung bean seeds before germination for the safety of mung bean sprouts.

P3-95 Risk Management of *Bacillus thuringiensis* Use in Agriculture – Leveraging an Important Biological Pesticide to Help Ensure Global Food Security

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Introduction: *Bacillus thuringiensis* has been successfully used as a biological pesticide in crop production for over 60 years. Now there is concern that *B. thuringiensis* may be linked to diarrheal syndrome in humans, but with *B. thuringiensis* accounting for up to 90% of the biological pesticides currently on the global market, it has a crucial role to play in continuing to support global food security.

Purpose: Education and risk management approaches for use of *B. thuringiensis* in crop production.

Methods: Review of publicly available information, including regulatory documents to understand the link between *B. thuringiensis* and diarrheal syndrome; identification of regulatory implications for food and crop production and supply; proposition of risk management approaches.

Results: Given the widespread use of *B. thuringiensis* in agriculture and food production globally, it is unlikely that ingestion of the microorganism causes a substantial number of foodborne illness outbreaks. It is more prudent to carefully determine and manage the risk of foodborne outbreak from *B. thuringiensis* strains than to completely remove *B. thuringiensis*-based or treated products from the market. However, assessing the risks of *B. thuringiensis* at the strain level requires a shift to a more strain-specific approach, focusing on pathogenicity traits and virulence factors. More recent technologies

for unequivocal strain typing, pathotyping, toxin-related gene profiling and toxin quantification offer promising novel approaches for food microbiology diagnostics and consumer risk assessment.

Significance: By being able to assess the potential risks from detection of *B. thuringiensis* in food or crop produce, manufacturers and growers may be able to avoid unnecessary disposal of food or crop produce and loss of profit. Moreover, they will also be able to put adequate agricultural and food production practices in place to mitigate the risk potentially associated with *B. thuringiensis* contamination.

P3-96 Control of *Staphylococcus aureus* and *Clostridium perfringens* during Smoke and Stabilization Cycle in Partially Cooked Bacon Processing

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Introduction: It is necessary to verify the control of *C. perfringens and* S. *aureus* in partially cooked meat products such as bacon because existing FSIS guidelines are intended only for ready to eat meats. FSIS safe harbor guidelines have been commonly cited by bacon manufacturers as scientific justification for control of spore-forming toxigenic bacteria including *C. perfringens* during stabilization. Additionally, outgrowth of *Staphylococcus aureus* during smoke cycle of bacon is a concern because temperatures necessary for lethality are not achieved and the product remains within the growth range of the organism for extended periods of time.

Purpose: To determine the survival of C. *perfringens* and S. *aureus* during smoke and stabilization cycles in partially cooked bacon production.

Methods: Pork bellies cured with four different brines were inoculated on the surface and sub-surface with *S. aureus* and *C. perfringens* at 10^{6-7} CFU/cm^2. The bellies were subjected to smoke cycles in a smokehouse and stabilization cycles in a water bath. Samples were taken at regular intervals during the smoking and chilling process. C. *perfringens* was enumerated on tryptose sulfite cycloserine and egg yolk emulsion and incubated at 35°C anaerobically for 48 h. *S. aureus* was enumerated on Baird-Parker agar with egg yolk tellurite emulsion and incubated at 37°C for 48 h.

Results: S. *aureus* declined in both surface and sub-surface samples on all four brines during smoke and stabilization cycles. S. *aureus* decline ranged from -0.58 log to 3.8 log. C. *perfringens* showed increase of 0.95 log on one of the brines, and a decline of up to 1.8 log on three other brines.

Significance: This study shows that partially cooked smoked bacon processing presents minimal food safety hazards with no significant growth of S. *aureus* and C. *perfringens* during smoke and stabilization cycles.

P3-97 Validation of Carrot Muffin Baking Process to Control *Salmonella* Contamination

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Introduction: *Salmonella* has a remarkable ability to survive in raw ingredients such as wheat flour under dry conditions for lengthy periods of time. Therefore, effective inactivation of *Salmonella* during carrot muffin baking process is vital to ensure the safety of finished food products.

Purpose: To validate the carrot muffin baking process as an effective kill step to control *Salmonella* contamination.

Methods: The wheat flour was initially mist inoculated with a five serovar-cocktail of *Salmonella* and dried back to pre-inoculation water activity (a_w) level. In a stand mixer, 40 ml of water and 360 g of white sugar were mixed at low speed. Then, premix 1, egg whites, canola oil, water and invert sugar were added and mixed. Later, shredded carrots and inoculated premix 2 were added to prepare the muffin batter. The muffin batter was weighed (135 g/ liner) and placed into the muffin lining. Two sets of experiments were carried out one with fresh batter and the other one with overnight frozen batter. Prior to baking, 1.5 g of streusel topping was sprinkled on each muffin and muffins were baked at 300°F for 45 min, followed by 15 minutes of ambient cooling. Muffin samples were collected at 0, 9, 18, 27, 36, 45 and 60 min for *Salmonella*, a_w and pH analysis. *Salmonella* populations in muffins batter during baking and post-baking muffin samples were enumerated on injury recovery (BHI agar over-layered with XLD agar) media.

Results: In both the studies (frozen and unfrozen batter), the carrot muffins baking parameters demonstrated a >5 log reductions in *Salmonella* populations. The a_w decreases significantly during the baking process but the pH remains unchanged.

Significance: This study validates that baking carrot muffins at 300°F for at least 45 minutes will result in a >5 log (CFU/g) reduction in *Salmonella* populations, ensuring the safety of final food products.

P3-98 Far-UVC Light for Inactivating Foodborne Pathogens in a Liquid Medium and on Food-Contact Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Ultraviolet C (UVC) light treatment is an FDA-approved method for decontamination in the food industry. However, conventional UVC systems, which emit light at a wavelength of 254 nm, are known to be harmful to human skin and eyes and are therefore typically operated in places where human exposure is limited. In contrast, far-UVC light — defined as wavelengths of 200-225 nm — has been shown to be harmless to mammalian cells, yet still germicidal. Therefore, studying far-UVC's bacterial inactivation efficacy under various conditions is potentially of great importance to future decontamination applications.

Purpose: To investigate the efficacy of a microplasma-based far-UVC lamp (222 nm) for inactivating foodborne pathogens in a liquid buffer and on food-contact surfaces.

Methods: The researchers prepared 5-6 log CFU/ml suspensions of two foodborne pathogens, *Listeria monocytogenes* and *Escherichia coli* O157:H7, as a representative of Gram-positive and Gram-negative bacteria, respectively. To test the decontamination efficacy of far-UVC light, samples of phosphate-buffered saline and two of the most commonly used food-contact surfaces — i.e., polyethylene terephthalate film and stainless-steel coupon — were inoculated with both bacterial suspensions and treated for up to 10 minutes with a far-UVC lamp at a power ranging from 0.297 to 0.445 mW/cm^2 (as measured from its genomic center).

Results: The far-UVC lamp achieved at least five-log reductions of *E. coli* O157:H7 and *L. monocytogenes* in the liquid buffer and on both food-contact surfaces within 5 minutes. The inactivation efficacy of far-UVC light against both bacterial strains was also found to be enhanced as the UV dose increased, in power and/or treatment time.

Significance: This study demonstrates the potential of using far-UVC lamps for decontamination in the food industry, potentially even when workers and consumers are present.

P3-99 Application of Room Temperature Plasma to Eliminate *Listeria monocytogenes* Contamination on Food Processing Surfaces

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Introduction: Contamination of food processing surfaces with *Listeria monocytogenes* can cause food safety outbreaks and recalls with significant human health and economic consequences.

Poster

Purpose: Research was conducted to evaluate the efficacy of room temperature plasma (RT-plasma), a new technology that uses energy and gases, to eliminate *L. monocytogenes* on food processing surfaces.

Methods: Three common food processing surfaces (Neoprene, Polypropylene, and stainless steel) were cut into 5 squares each (2.5 cm by 2.5 cm) and inoculated with 5 logs of *L. monocytogenes* and placed at 4˚C for 30 minutes to allow cell attachment. Then, each sample was exposed to the RT-plasma generated using helium gas and an electrical input of 5.75 kW. After exposure for (0, 10 and 20 min), each sample was rinsed in buffer peptone water (10 mL), serially diluted and then streaked on Modified Oxford Agar plates which were incubated for 24 h at 37°C. After 24 h, typical *Listeria* colonies were counted and reported as log CFU/sq.cm the plates were counted.

Results: The experiment was repeated three times and the data was analyzed using ANOVA with Tukeys HSD ($P<0.05$) to find significant differences among the means. RT-plasma reduced ($P<0.05$) the population of *L. monocytogenes* on neoprene by 1 and 2 log after 10- and 20-min exposure, respectively. However, polypropylene and stainless-steel samples, did not demonstrate any reductions at 10 min but had a 2-log reduction after 20 min exposure.

Significance: These results demonstrate that RT-plasma can be used as a method to eliminate *L. monocytogenes* contamination on food processing surfaces.

P3-100 Inactivation of *Bacillus cereus* in Biofilm on a Stainless Steel Surface by Treatments with Gaseous Chlorine Dioxide

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❖ ***Developing Scientist Entrant***

Introduction: Bacillus cereus is known to form biofilm on a stainless steel surface and the biofilm forming on food-contact surfaces may cause microbial contamination in the food manufacturing environment.

Purpose: The objective of this study was to inactivate *B. cereus* in biofilm on a stainless steel surface using gaseous chlorine dioxide (ClO_2).

Methods: Each stainless steel (SS) coupon inoculated with *B. cereus* (approximately 7.1 log cfu/coupon) was incubated at 12 and 25°C for 4 days and 1 day, respectively, to form biofilms. Gaseous ClO_2 was generated from 0.5 or 1.0 mL of ClO_2 solution in air-tight containers at 25°C and concentrations of ClO_2 gas were monitored. SS coupons containing *B. cereus* in biofilm were exposed to gaseous ClO_2 at 25°C (RH 100%) for up to 6 h. SS coupons were vortexed in 30 mL of PBS or DE broth with glass beads, the suspension was surface plated onto TSA plates, and the plates were incubated at 30°C for 24 h. After the incubation, colonies on the plates were enumerated to determine population of *B. cereus*.

Results: The concentration of gaseous ClO_2 peaked (372 and 696 ppm) within 30 min and decreased to 204 and 408 ppm after 6 h, when the gas was generated from 0.5 and 1.0 mL of ClO_2 solution, respectively. Populations of *B. cereus* in biofilms forming at 12°C for 4 days and 25°C for 1 day were 5.3 and 5.6 cfu/coupon, respectively, and the organisms in the biofilms were completely eliminated within 30 min of the gaseous ClO_2 treatment, regardless of concentration of ClO_2 gas. However, 3.6 - 4.4 log cfu/coupon of *B. cereus* still survived in biofilm on untreated SS coupons after 6 h.

Significance: These results indicate that gaseous ClO_2 has antimicrobial activity against *B. cereus* in biofilm on a stainless steel surface.

P3-101 A Real-Time Nondestructive Food Quality Monitoring System Based on Paper Chromogenic Array and Machine Learning

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Introduction: Shifts in color patterns of the paper chromogenic array can be analyzed and revealed by machine learning models, reflecting changes in food VOCs and thus tracking food quality.

Purpose: The purpose of this study was to build a low-cost, real-time, and nondestructive food quality monitoring system.

Methods: A standardized 4x4 paper chromogenic array was fabricated by photolithography and paper microfluidics with 16 chromogenic dye spots, costing less than $0.9 per unit in the lab (even lower for industrial production). The quality of 36 fish samples under different conditions of temperature (abused, refrigerated, and ambient), microbial contamination (*E. coli*, *Listeria monocytogenes*, *Salmonella* spp.), and days of storage (0, 1-3 fresh, 5-7 semi-fresh, 7+ spoiled) was analyzed and tracked by an advanced neural network with the extracted RGB dataset from scanned images of the paper chromogenic array after exposure. A 6-fold Cross-validation and metaheuristic algorithms (PSO and GS) were used for the fine-tuning parameters of the neural network.

Results: For all 36 fish samples, the multi-layer neural network was able to identify differences in color pattern changes in the paper chromogenic array between samples contaminate by monoculture and cocktail culture of pathogens under ambient and refrigerated temperatures ($P<0.05$). The category of the freshness of fish samples was predicted at accuracy rates of 100% in the training set and 91% in the testing set.

Significance: Paper chromogenic array has the potential to be integrated into smart food packing at a very low cost. Real-time nondestructive food quality monitoring is achieved by monitoring the changes in color patterns and obtaining rapid feedback by edge computing.

P3-102 Examination of the Use of Failure Mode and Effects Analysis (FMEA) to Improve the Risk Assessment of Biological Hazards of a Fresh-Cut Produce Processing Plant

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Introduction: Fresh-cut produce is a source of foodborne outbreaks, especially when fresh-cut processors fail to recognize when their biological hazards are not adequately controlled because of errors in risk assessment, thus leading to Type-2 errors. It is suggested that these outbreaks continue to occur because the risk associated with processing is not being comprehensively portrayed by a conventional Hazard Analysis (HA).

Purpose: This study compares FMEA, a risk assessment methodology employed by many other non-food manufacturing sectors, with a conventional HA to assess the relative risks of biological hazards on a fresh-cut carrot processing line.

Methods: A conventional HA was performed in which biological hazards were identified for each process step after which each hazard was determined to be either significant or not. This HA was compared to a FMEA where potential biological failure modes were identified at each process step after which risk priority was calculated from the severity, occurrence and detection ratings associated with each potential FMEA failure mode and attendant process controls. This information was then used to determine residual risk and prioritize the process steps requiring further corrective actions.

Results: The HA suggested that properly peeling and washing carrots from approved suppliers will adequately control the pathogen load while the FMEA results suggested there remains some residual risk in fresh-cut carrot processing largely because there are currently no realistic methods for detecting pathogens in the incoming carrots.

Significance: FMEA, in contrast with HA, provides a more precise picture of the residual risk associated with a fresh-cut produce processing line. This information can then be used by the processor to focus on continuous improvement activities that will lead to decreased risk, thereby reducing the likelihood of a foodborne outbreak.

P3-103 Mitigation of *Salmonella* in Ground Pork Products through the Physical Removal of Tonsil Glands and Lymph Nodes in Pork Trimmings

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❖ ***Developing Scientist Entrant***

Introduction: Previous bio-mapping studies of pork processing facilities have indicated a need for mitigation strategies in trim and ground products due to an increase in *Salmonella* prevalence. Lymph nodes have been identified as a source of *Salmonella* in pork products.

Purpose: The objective of this study was to determine if the removal of glands and lymph nodes in pork would reduce *Salmonella* and indicator organisms in comminuted products.

Methods: Samples from a commercial pork facility were divided into three treatment groups; 1) untreated control, 2) topical glands removed before final processing, and 3) topical, jowl, and internal lymph nodes and glands removed before final processing. APC and EB were enumerated with the BioMérieux TEMPO® system. Samples were evaluated for the presence and quantity of *Salmonella* using the BAX® System Real-Time *Salmonella* SalQuant™ methodology. This study was conducted for 5 replications over 4 months to account for microorganism natural variation, resulting in a total sample size of 450.

Results: The results of this study indicate that the removal of the topical, internal, and jowl lymph nodes was effective at significantly ($P > 0.05$) reducing the prevalence of both *Salmonella* and the indicator organisms evaluated in further processed pork. 52.7% of samples were suitable for *Salmonella* enumeration, the majority from the control group. *Salmonella* was reduced from 2.5-Log CFU/Sample and 3.8-Log CFU/Sample of *Salmonella* in control group ground and trim samples, to less than 1-Log CFU/sample in both matrices from treatment 3.

Significance: This indicates that samples from treatment 3 showed an average of a 3-Log reduction, and treatment 2 showed a 2.5-Log reduction, of *Salmonella* when compared to the control samples. This indicates that the physical removal of glands and lymph nodes can influence *Salmonella* and indicator organism prevalence in the final product, however, further research is needed to determine node removal feasibility in processing environments.

P3-104 National Surveillance of Microbial Indicators and Foodborne Pathogens in Commercial Beef Processing Facilities in a Central American Country

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Introduction: Central American countries have expanded their exports of meat to different parts of the world, and national surveillance systems are an integral part of equivalency of inspection negotiation between trading partners. As standards continue to become more demanding, food safety management through microbial surveillance provides an access for data-driven decisions in the processing chain.

Purpose: Evaluate the loads of microbial indicators and pathogen prevalence in five different commercial beef processing facilities in a Central American country to develop a baseline benchmark for processors interested in accessing export markets.

Methods: Samples were collected by trained inspection personnel throughout a 36-month period of operations to account for process, lot, and facility variability. Carcass swabs were collected at different processing steps (whole carcass (WC), carcass cuts (CC), and ground beef (GB)). The samples were analyzed for Aerobic Count (AC), generic *Escherichia coli*(EC) and coliforms (COL), *Staphylococcus aureus* (SA)*,* and Fungi and Yeasts (FY). Samples were also tested for prevalence of *Salmonella* spp., Shiga-toxin Producing *Escherichia coli* (STEC), and *Listeria monocytogenes*.

Results: Overall, samples had a very low prevalence result for *Salmonella* spp., 1/766 (0.13% ± 0.0013) throughout 3 years. Prevalence of *Listeria monocytogenes* was low with 1/154 (0.64% ± 0.0029). Additionally, 7 STEC positive samples from n = 473 (2.75% ± 0.0043) were detected. The enumeration of microbial indicators did not show statistical difference throughout the years for the facilities evaluated for AC, EC, YM counts and *Staphylococcus aureus*.

Significance: This study confirms that for three years of surveillance in different commercial processing plants from a Central American country, there was a low prevalence on pathogenic microorganisms showing effective implementation of their food safety systems. Low indicator microorganism concentration variations throughout the years show the maintenance of their microbial baseline within plants, further elucidating their commitment to maintaining low microorganism concentrations to ensure food safety and quality.

P3-105 Bio-Mapping of Pathogen Levels in a Cattle Processing Facility

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Introduction: To control carcass contamination is key to identify the sources of contamination along the process.

Purpose: Therefore, a bio-mapping study was performed to identify microbial population in samples taken antemortem for pathogens found in cattle at a commercial processing facility.

Methods: Prevalence of pathogens such as *Salmonella*, *Escherichia coli* O157:H7, and non O157:H7 shiga-toxin producing *Escherichia coli* as STEC panel 1 (serogroups O26, O111, O121), and STEC panel 2 (serogroups O45, O103, O14) were studied. Samples were collected at the lairage area and at hides. For lairage area, boot socks were used for sampling, samples consisted in five boot swabs taken each day from five different dirty pens, a total of 60 samples were collected. Moreover, for hide swabs, the sampled locations were foreshank-brisket (one swab for both locations) and inside round, 10 swabs were collected per day, in total 120 swabs were taken. The samples were tested using the BAX System, for the statistical analysis an ANOVA was performed ($p<0.05$), with a Tukey media separation. All the data used was free of outliers.

Results: The results presented a higher percentage of screen positives of *Escherichia coli* O157:H7 for boot swabs in comparison to hide swabs, as well boot swabs presented a higher percentage of screen positives of *Salmonella* with a value of 100%, for STEC Panel 1 and 2, both presented a similar behavior. In case of hides, the screen positives were lower in comparison to boot swabs for all pathogens, however, in between samples taken on foreshank-brisket and inside round, hide foreshank brisket presented a higher percentage for *Salmonella.*

Significance: As expected, the most contaminated area was the lairage area, were the prevalence of pathogens seemed to be higher, however when the cattle enter the processing facility there is a decrease in screen positives found in hides, which means interventions are working.

P3-106 Evaluation of Chemical Properties and Indicator Microorganisms Enumeration on Chicken Tenderloins

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Introduction: Poultry processors and food service operations must coordinate proper product handling to assure product quality, shelf-life, and safety from process to preparation and restaurant consumption.

Purpose: This shelf-life study was designed to evaluate the microbial and chemical shelf-life of refrigerated chicken tenders harvested by hand-deboning and mechanical deboning from processing to restaurant storage and preparation for integrated food safety and quality management optimization.

Methods: Chicken tenderloins were obtained from hand-deboning (cone-line) systems and mechanical de-boning (automatic harvester). The tenderloins were packed in 4 bags of 10-lb and then packed inside a modified atmosphere (N_2) in a secondary bag. Bags were kept under refrigerated conditions (4°C) for microbial and sensory analysis after 0, 3, 7, 14 and 21 days. A total of three repetitions were conducted in this study with a total of 15 (40-lb) bags. Mesophilic aerobic counts (AC), psychrotrophs (PSY) and anaerobic lactic acid bacteria (LAB) were enumerated using the TEMPO® System. In addition, pH analysis was performed at 0, 7, 14, and 21 days of storage under refrigerated conditions (4°C).

Poster

Results: As expected, PSY, AC and LAB counts showed a statistically significant difference when comparing different days of storage (P< 0.001), with increases of 3.8 logCFU/ml, 2.9 log CFU/ ml and 2.8 logCFU/ml, respectively. Day 21 of storage indicated extensive counts in PSY with 9.2 logCFU/ml, AC with 8.2 logCFU/ml and LAB with 6.3 logCFU/ml. The samples showed an average in pH from 6.17 in day 0 to 5.94 in day 21.

Significance: Shelf-life studies accounting for microbial indicator growth and chemical product changes during the distribution, storage, and preparation of poultry products from different deboning systems can serve processors and food service operations to identify best practices and potential interventions that can extend product quality and shelf-life in fast food settings and reduce food waste from spoiled products.

P3-107 Surveillance of *Listeria monocytogenes* in Chicken Production for Export during 2020–2022

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***Introduction:* ,** *Listeria monocytogenes* is a Gram-positive, facultatively anaerobic, rod-shaped pathogenic bacterium that can cause listeriosis, a serious infection. Implementing a comprehensive surveillance program can help ensure that chicken products are free from *L. monocytogenes* and are safe for export and consumption.

***Purpose:* ,** The aim of this study was to detect and prevent the contamination of poultry products with *L. monocytogenes* in chicken production and implement control measures to prevent further contamination.

***Methods:* ,** A total of 4,420 samples were collected from chicken production facilities during 2020-2022. These samples included raw chicken products, cooked chicken products, utensil swabs, and gown and apron swabs (n = 102, 120, 3,928, and 270 tested samples, respectively). These samples were analyzed using enzyme-linked immunosorbent assay (ELISA) technology to detect specific proteins produced by *L. monocytogenes*. Logistic regression models were expressed as odds ratios with 95% confidence interval.

Results: , The results showed that *L. monocytogenes* was found in 0.29% (4/1,393), 0.27% (4/1,505), and 0.53% (8/1,522) of the samples in 2020, 2021, and 2022, respectively. There was no significant difference in contamination between raw and cooked chicken products (p>0.05). The contamination levels in these products were higher than in utensil swabs (p<0.05), and *L. monocytogenes* was absent in gown and apron swab samples.

***Significance:* ,** These findings can be used to identify areas for improvement in efforts to reduce *L. monocytogenes* contamination in chicken production.

P3-108 Detection of *Salmonella* Contaminated Poultry Products Using a Commercial Tissue Dissociation System

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Introduction: While significant advancements have been made in rapid detection methods, the process of bacterial separation remains a bottleneck for pathogen detection in food matrices.

Purpose: A commercial system for mechanical and enzymatic digestion of skeletal muscle was evaluated to detect the prevalence of *Salmonell*a in raw poultry using conventional laboratory screening diagnostics.

Methods: This study benchmarked a tissue digestion system, GentleMACS (Miltenyi), which applies both chemical and physical methods to separate microorganisms from tissues, against stomaching, a current standard utilized by commercial and regulatory food safety laboratories. The impact of the treatment on the physical properties of chicken thighs with skin was characterized using a weight-based degradation assay in combination with dynamic light scattering. influence of the treatment on *Salmonella* detection was assessed using sample preparation consistent with the Microbiology Laboratory Guidebook (MLG-4) as set forth by the USDA Food Safety and Inspection Service (FSIS) in triplicate using microbiological plating, quantitative PCR (qPCR), and loop-mediated isothermal amplification (LAMP).

Results: The results indicate the tissue digestion system has more significant impact reducing the average particle size of the matrix relative to stomaching (P<0.001). Real-time PCR and 6x6 drop plate assays used to detect *Salmonella* were not adversely affected by the treatment. Furthermore, inoculated chicken treated with the GentleMACS resulted in a significant increase (P<0.003) in the platform's detection capabilities relative to stomached controls. Cohen kappa coefficient and McNemar's test indicate the 6x6 and PCR results agree with measurements obtained via the 3M Molecular Detection System (κ>0.62; P>0.08).

Significance: While further research is necessary to apply this technique to practical detection, this work demonstrates the potential for commercial tissue digestion systems to identify pathogens in meat products that are otherwise difficult to detect. Additional work is required to scale the procedure in order to accommodate all of the sample sizes/volumes used in USDA FSIS sampling protocols.

P3-109 Establishment of Co-Culture Models of the Human Intestinal Epithelium to Assess Gut Barrier Functions after Exposure to Emulsifiers and Live Microbials

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Introduction: Dietary components have the potential to regulate gut barrier integrity and functions. The interaction of food components (emulsifiers and live microbials) with the intestinal epithelium and the subsequent responses have not been thoroughly evaluated**.**

Purpose: To establish co-culture models that simulate the intestinal epithelium for better assessing the responses to mixed-food components.

Methods: Human intestinal epithelial cells (Caco-2: goblet HT-29-MTX) were mixed to represent small (90:10) and large (75:25) intestinal barriers. Monolayers of Caco-2, HT-29-MTX, and co-cultures were treated with emulsifier polysorbate (P)-80, and live microbials: *Lactobacillus acidophilus* (LA), *Streptococcus thermophilus* (ST), and *Bacillus subtilis* (BS). The trans-epithelial electrical resistance (TEER), lucifer yellow flux, cellular viability, and expression of biomarkers (tight junction, mucin, and cytokines genes) were examined.

Results: Upon treatment with live microbials at a multiplicity of infection (MOI) of 10:1 and 20:1, the TEER values for all monolayers markedly increased with LA but did not change with ST or BS. (P)-80 (0.001%-0.2%) alone resulted in a dose dependent decrease of TEER in treated Caco-2 and 90:10 mixed monolayers, while HT-29-MTX and 75:25 mixed monolayers were less responsive, as shown by lucifer yellow flux and cell proliferation. Following recovery experiments, the integrity of monolayers was restored with proliferating cells. Except for claudins, there were no significant differences in the expression of the tight junction genes of microbials treated co-culture monolayers. Differential expression of mucin and cytokine genes were observed between microbial treatments in co-culture models. Noticeably proinflammatory cytokine CXCL8 was downregulated when treated with LA in both co-culture models.

Significance: Preliminary data indicate that presence of live microbials do not adversely affect the integrity and functions of the epithelium but there are differences in their potencies. These established co-culture models allow us to further investigate the impact of live microbials combined with emulsifiers on the gut mucosal responses.

P3-110 The Role of Traditional Markets in Ensuring Food Safety in Products from the Horticultural Sector in Ethiopia

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Introduction: Traditional markets are common in many parts of Ethiopia where millions of people purchase fresh horticultural produce, animal products, grains, and other food commodities, although fruits and vegetables in these markets have been found to be contaminated by bacteria such as**.** E. coli strains, Staphylococcus aureus enterotoxin and *Salmonella* spp.

Purpose: The purpose of this review is to highlight the food safety situation of horticultural products in traditional markets in Ethiopia and discuss ways of addressing the food safety risk resulting from the marketplace.

Methods: This review was based on a survey of 30 articles and reports and discussion with 6 experts from Ministry of Agriculture on food safety of horticultural products in Ethiopia.

Results: Most horticultural products in Ethiopia are sold in traditional markets where products can be cross contaminated by pathogenic bacteria. Traditional markets have different food safety challenges such as low awareness of food safety risks by vendors, unclean environments, poor waste handling, lack of clean water as well as the absence of regulations. Addressing these gaps at a market level can contribute to reducing food safety hazards and minimizing foodborne disease.

Significance: Traditional markets have a role in preventing food safety risks. The discussion on the risks and ways to improve traditional markets can draw attention to addressing the challenges of food safety in traditional markets.

P3-111 Consumer Perception of Street Foods Safety in Lagos, Nigeria

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Introduction: Street foods are known to contribute significantly to the informal food distribution sector and meet the challenges of food security for all socioeconomic classes. However, the potential foodborne diseases and associated illnesses in the consumption of street foods are concerning and worthy of study. The safety status of street food is of immense importance most importantly in the urban and densely populated areas such as the city of Lagos, Nigeria which has a population density of close to 20 million residents.

Purpose: The apparent poor nutritional composition and associated food safety hazards of street foods are concerning. This study, therefore, investigated the perceived customer attitudes towards the safety of street foods in the selected local government of Lagos state, Nigeria. Participants were drawn from four (4) Local Government Areas in Lagos state, Nigeria (Mushin, Ikeja, Agege, and Oshodi/Isolo).

Methods: A survey was deployed to 135 participants in the selected area The questionnaire was administered to consumers to determine their consumption patterns, frequency, and health effects.

Results: 78 % of the participants admitted to consuming street foods at least once a day.71% of the participants are comfortable consuming street foods. 77% of the participants have no fear of consuming street foods. However, 83% of the participants reported being sick at least once from consuming street foods. 78% of the participants believe that training, knowledge, and education about proper food handling are absent in street food vendors. 97% of the participants agree that government regulation and supervisory oversight is necessary

Significance: This study uncovered that while street food continues to be a ready source of nutrition for many consumers, and consumers are willing to continue patronizing street food vendors, the need for the government to inaugurate regulations and operating standards for street food to reduce associated health and safety hazards is necessary.

P3-112 The Cost of Diarrheal Illnesses in Ethiopia

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Introduction: Diarrheal illnesses are prevalent in Ethiopia and can lead to severe complications and costs that threaten nutrition for already impoverished households.

Purpose: This study uses survey data collected by our study team in Ethiopia to estimate the cost of diarrheal illnesses to households and society.

Methods: Using a community-based survey conducted in three population centers in Ethiopia (Addis Ababa, Gondar, and Harar), we obtained an initial sample of 2,438 households, with 247 reporting at least one diarrhea case over the previous four weeks (omitting 5 observations with extreme value, implausible answer, and missing value).We then computed preliminary estimates for the self-reported cost of diarrheal illnesses based on the most recent diarrhea episode in the household over the last four weeks. The total self-reported cost is estimated based on the cost of healthcare provider visits, travel and lodging expenses, medicine and other treatment cost, loss of productivity for those made ill and their caregivers, and other costs that are not included in the categories described above.

Results: Our preliminary estimate for average self-reported costs is 294.99 (s.d.1,351.88) Birr ($5.48) per episode. The average self-reported costs for healthcare provider visit, travel, transportation, and lodging, medicine and treatment, and other items are 14.18 (s.d. 15.65) Birr, 22.63 (s.d. 53.03) Birr, 124.93 (s.d. 294.72) Birr and 11.70 (s.d. 63.38) Birr respectively. The average loss of productivity for those ill is 24.72 (172.37) Birr and average loss of productivity for caregiver is 96.82 (s.d. 1278.80) Birr. Medicine and other treatment costs account for the largest share of self-reported costs (42.35%).

Significance: The derivation of improved estimates of the cost of diarrheal illnesses in Ethiopia could inform government policy and provide incentives for the government to allocate more resources to mitigate the severe negative impacts of diarrheal illnesses.

P3-113 A Conceptual Framework for Food Safety Interventions: Insights from Low- and Middle-Income Countries

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❖ ***Developing Scientist Entrant***

Introduction: Historically, a variety of interventions to improve the safety of fresh foods have been conducted in low- and middle- income countries (LMICs), including provision of infrastructure; awareness raising; and introduction and training in new technologies and processes. The three-legged-stool model (Johnson, 2015; Grace, 2019) describes three critical factors for an intervention to be efficacious: An enabling environment; training and technology; and incentives for intervention beneficiaries. However, very few studies have reported on aspects beyond efficacy that indicate success, such as cost, scalability, sustainability, equity, and cultural/geographical context.

Purpose: This study aims to develop a conceptual framework highlighting major themes around food safety initiatives and how they contribute to its success measured across multiple domains.

Methods: Focus Group Discussions (n= 16) and Key Informant Interviews (n= 9) were conducted in Nigeria and India along with a literature review. A combination of inductive and deductive coding through NVivo led to identification of major themes and their relationships.

Results: The enabling environment, training & technology and incentives, remain core contributors to an intervention's efficacy in LMICs. Nevertheless, there exist pre-requisites and alleviators contributing to the sustainability and scalability of a campaign. A pre-requisite standard of living, education and available utilities support early adoption of safe practices beyond an original intervention group, allowing wider roll-out (scalability). Respondents (dairy farmers & traders) in India voiced distress caused by limited land, cattle illness, water availability being a bigger concern than selling hygienic milk. Moreover, alleviators such as affordability, accessibility, value addition and integration with traditional beliefs contribute to campaigns being sustained beyond a research project lifespan.

Significance: The current framework brings together a globally applicable perspective and relationships between the emerging concepts to guide future policy and practice around food safety initiatives. This will also encourage reporting of indicators beyond efficacy to measure intervention success.

P3-114 Identifying Predictors of Safe Food Handling Practices among Canadian Households with Children Under 18 Years

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❖ ***Developing Scientist Entrant***

Introduction: Poor food handling practices at home are a common cause of foodborne illness. Children are the most susceptible population group for foodborne illness compared to adults.

Purpose: This study aimed to measure the prevalence of food safety practices of primary food preparers in Canadian households with children (<18 years old) and assess if they are associated with sociodemographic factors.

Methods: Data for Canadian households with children (n=294) were extracted from a larger telephone survey conducted across all Canadian provinces and territories between 2014 – 2015. One question representing each of the core food safety practices (clean, cook, chill, and separate) was identified based on prior evidence and relevance to families with children. Four food safety practice outcomes and six demographic variables were examined using multivariable logistics regression models.

Results: Most survey participants were females (56%), with less than a bachelor's degree (67%), and caring for one child (vs. two or more) under 18 years (55%). Approximately 90% of caregivers reported proper hand hygiene after handling raw meat, and 79% refrigerated leftovers within 2 hours of cooking. Only 33% of caregivers reported taking steps to prevent cross-contamination of raw meat in the refrigerator. The use of a food thermometer to check cooking doneness varied by meat type; 36% of respondents reported using a thermometer for whole chicken, but fewer reported using thermometers for poultry cuts (13%) and ground meat such as hamburgers (11%). Respondents from higher income and education categories were less likely to follow safe food handling practices such as hand hygiene and safely refrigerating leftovers. Better food handling outcomes were observed among female participants for safe food storage and among respondents from households in urban areas for food thermometer usage.

Significance: This research highlights the need for food safety interventions targeting Canadian families with children within certain demographic groups.

P3-115 Addressing Listeriosis – A Challenge in Direct-to-Consumer Food Establishments

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Introduction: In Canada, listeriosis is the leading pathogen-associated cause of death for food-borne illness each year. In the past 10 years, Ontario has seen a number of listeriosis outbreaks, however identifying the root cause of these outbreaks is challenging.

Purpose: Following outbreaks in multiple direct-to-consumer (DTC) food establishments (e.g. long-term care homes, restaurants), and failure to identify root causes in many cases, Public Health Ontario (PHO) performed an evidence search on best practices for outbreak management, specifically for identifying *Listeria* harborage or colonization sites, developing strategies for sampling, remediation and preventative measures, to provide assistance for investigation and identification of root causes.

Methods: To identify information on sampling strategy and remedial and preventative measures for listeriosis outbreak management in DTC food establishments, a literature search of three databases was performed. A custom grey literature search using Google and Google Scholar was conducted. Information was abstracted from articles that were relevant to DTC food establishments. A summary of results will be included in a document published by PHO.

Results: Search results will be presented, focusing on practical information for environmental investigations during listeriosis outbreaks. Risk factors associated with *Listeria* in food establishments (e.g., cross contamination), identifying common sites where *Listeria* can be found to assist with the development of a sampling strategy, biofilm identification and control, cleaning, sanitizing (e.g., rotating sanitizers to prevent the formation of resistant listeria), hurdle concepts effective at eliminating and preventing *Listeria* and other mitigation strategies, are some of the examples that will be discussed.

Significance: This review will assist outbreak investigations by providing evidence based actions for identification, mitigation and prevention for listeriosis outbreaks in DTC food establishments. Education and awareness of risk can assist in reduction of risk and ultimately reducing the burden of illness.

P3-116 Prioritising Food Safety Culture Measures to Generate a Bespoke, Food Manufacturing Industry Appropriate Tool

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Introduction: Annually, data shows 10% of the global population acquires a foodborne-disease indicating food-safety(FS) continues to be a public-health challenge. A positive food-safety-culture(FSC) is considered to be the foundation of robust FS management systems and FSC measurement/improvement is a legislative and Global-Food-Safety-Initiative requirement for food/drink manufacturing businesses. Commercial measurement-mechanisms may be prohibitively costly, whilst bespoke measurement-mechanisms developed with appropriate expertise may specifically address business needs and be more financially viable.

Purpose: To rationalise a bespoke, quantitative FSC-questionnaire to enhance practical feasibility within a low-risk food manufacturing business.

Methods: To prioritise/condense attitude-statements related to FSC-components, intercorrelations between attitude-statements within questionnaire sections were determined using Kendall's Tau-b co-efficient. Where significant correlations were found, statements were removed, then questionnaire coded-scores were compared pre and post-rationalisation using the Mann-Whitney U test to ensure that removal of the statement did not significantly change(<0.05) overall questionnaire coded-scores.

Results: Cumulatively, 23% of statements were rationalised from FSC-questionnaire which took 22-minutes on average, it was ensured that all FSC-components were addressed post-removal. Within FSC-component metrics, determination of significant correlation(tau=634,p<0.01) between perceived motivation/perceived need for increased communication of FS-metrics meant an attitude statement could be removed. The Mann-Whitney-test indicated removal of the statement had no significant effect on median score(3.58 to 3.52). Implementation of revised rationalised questionnaire showed reduction in completion time by 6-minutes and increased operative response-rate(11.1%).

Significance: Statistical rationalisation of FSC measurement-mechanisms allows for academic and industry needs to be satisfied, whilst not impacting FSC measurement/improvement data. Industry benefits include; improved business-perception toward FSC measurement, increased participation and management-commitment, ultimately contributing to improvement identification within businesses thus improving FS-standards.

Poster

P3-117 Cognitive Progression in the Alignment of Assessment Results with Effective Interventions Toward Improving Food Safety Culture

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Introduction: Contemporary methodologies for assessing food safety culture (FSC) utilizes triangulation (surveys, interviews, focus groups) linked to findings from observations and peripheral audit and QC documentation. Interpreting and concluding on the findings, and effectively linking with targeted strategies for improvement while limiting assumptions and generalizations, has proven complex and sometimes ineffective, demanding expenditures toward FSC improvement interventions, without reaping the desired reward.

Purpose: The current study explores the value of progression of cognitive arguments accompanying research results obtained through FSC assessments and proposes future best-practice.

Methods: A meta-analysis was performed on a broad selection of FSC assessment data across a wide range of food production and -service facilities including retail, diary, fresh produce, canned food, and hospitality, and the articulation amongst descriptive and inferential statistics, narratives and arguments toward tangible implementation were investigated. The role of selected cognitive categories such as "explain", "discuss" and "conclude" populated by a broader selection of verbs from Bloom's Taxonomy is further alluded to, and a chain of thought to ensure tangible conclusions and recommendations, linked with effective interventions are proposed.

Results: The results from this study indicates that: 1) A strong case can be made for the identification and utilization of selected cognitive verbs in identifying targeted and realistic FSC improvement interventions; 2) the level of cognitive argument in comparison to the volume of empirical results collected from FSC surveys are customarily unbalanced and; 3) skills development amongst FSC consultants, academia and industry specialists toward an understanding of cognitive progression and its application in empirical data interpretation is essential to ensure the advancement of the FSC discipline.

Significance: The study aims to limit qualitative and quantitative observations that may have led to generalizations and assumptions on FSC, resulting in skewed feedback and recommendations to industries.

P3-118 Development of a Framework to Capture the Maturity of Food Safety Regulatory and Enforcement Agencies: Insights from a Delphi Study

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Introduction: Currently, global food safety regulatory and enforcement agencies have no standardised means of assessing their effectiveness and the maturity of their national food safety auditing and inspection systems - this has an impact on the quality and safety of food within global supply chains.

Purpose: The purpose of the study was to present the first stage of work being undertaken to develop and evaluate a maturity framework designed to assess and benchmark the effectiveness, ability to achieve continuous improvement, and optimise processes and functioning of food safety regulatory and enforcement agencies across the world.

Methods: To achieve this aim, a comparison of global food safety regulations, and Delphi-interviews with stakeholders of food safety regulatory and enforcement agencies from Australia, Canada, Ireland, and USA were carried out. Through inductive, textual data analysis, three dimensions and thirteen sub-dimensions were identified that covered cultural and systems elements influencing the quality and impact of food safety regulations across the world as well as the gaps identified by the stakeholders.

Results: The findings of the study are that whilst there is broad support by food safety regulators for developing a benchmarking and evaluation framework for food safety regulatory and enforcement agencies, there are also some outstanding challenges such as defining globally applicable measures, buy-in from specialised agencies and senior management to adopt a maturity framework to change the culture within regulatory agencies, and the role played by governments in influencing the efficiency and functioning of regulatory systems.

Significance: The study identifies the building blocks of global food safety regulatory and enforcement agencies as the first step towards the development of a maturity framework. The building blocks would help agencies across the world revisit the dimensions used to carry out internal audits and design continuous improvement projects, while helping new agencies establish themselves based on the building blocks.

P3-119 Development of an Automated Solid Phase Extraction Instrument for Determination of Lead in High-Salt Foods

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Introduction: Heavy metals are widely present in food products and food processing environment and can cause severe adverse effects on human health.

Purpose: In this study, an instrument for solid phase extraction of heavy metals was developed.

Methods: After analyzing the partition coefficient and adsorption capacity of the exchange resin, the Chelex-100 resin achieved a strong adsorption of lead at pH of 6.0. Compared to the matrix modifier method, solid phase extraction eliminated the interference of salts and the results were more accurate and reliable. The accuracy and stability of this instrument were verified by inter-laboratory testing of the quality control samples of soy sauce (CFA-PA-QC1728-4) between six laboratories.

Results: Compared to conventional separation equipment, the instrument developed in this study has advantages of small size, less reagent consumption, time saving, easy operation, and good reproducibility. In the inter- laboratory testing, the results from six laboratories were within the standard range with relative standard deviation (RSD) of 2.2%-2.8%. Recovery of three spiked samples was within 80.0%-100.0% and RSD was of 2.8%-9.3%.

Significance: The results suggesting that this instrument can be used to separate and enrich lead in high-salt foods.

P3-120 Gas Phase Hydroxyl-Radical Process for Decontaminating Hatchery Eggs: Improving Chick Health and Food Safety

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Introduction: Hatchery eggs can harbor human (e.g. *Salmonella*) and avian (e.g. Avian pathogenic *Escherichia coli*) pathogens on the surface that can be transferred and subsequently infect the developing embryo. This can result in the death of the embryo or carriage in the resultant chick. Current egg disinfection methods include formaldehyde fumigation which, although effective, is a worker hazard, time consuming, and can have negative effects of hatchery rate if not controlled.

Purpose: The following study developed and evaluated an alternative egg disinfection method based on gas-phase hydroxyl radical treatment. The objective was to optimize treatments to inactivate pathogens without negatively affecting hatchery rate or chick health.

Methods: The independent parameters of the gas phase hydroxyl radical treatment were UV-C dose, hydrogen peroxide concentration, and ozone concentration. The independent variables were the reduction of *Salmonella* and changes in egg quality metrics. The optimized treatment was applied to hatchery eggs and the hatchery yield along with chick development monitored over a 49 day grow out period.

Results: The optimized treatment that reduced *Salmonella* levels by 5 logCFU was 2% hydrogen peroxide, 20 ppm ozone, and 19 mJ/cm2 UV-C (equated to a five second treatment). The same treatment could also support the same level of inactivation of c*ampylobacter jejuni*, *Escherichia coli,* and *Pseudomonas aeruginosa,* which are commonly associated with eggs. The cuticle layer and inner egg membrane were not affected by the hydroxyl-radical treatment. Tri-

als within a commercial hatchery demonstrated that Enterobacteriaceae was reduced on treated eggs to below the level of detection (<1 log CFU) with no *Salmonella* being recovered. Moreover, trials using 7000 eggs demonstrated no negative effect on embryo development, hatchery rate, or chick outgrowth relative to formaldehyde controls.

Significance: Gas phase-hydroxyl radical treatment can be used to successfully inactivate pathogens of concern in hatchery operations, thereby providing an alternative to formaldehyde fumigation.

P3-121 Development and Validation of a Dynamic Predictive Model for Growth of *Bacillus cereus* in Turkey Roast

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❖ ***Developing Scientist Entrant***

Introduction: *Bacillus cereus* is a ubiquitous, psychrotrophic foodborne pathogen often isolated from several raw food materials and occasionally in processed foods and can cause diarrheal and emetic illnesses in humans.

Purpose: To develop and validate a dynamic predictive model for *B. cereus* in turkey breast roast under varying temperatures.

Methods: Turkey breast meat was ground and mixed with a marinade containing water, salt, and phosphate (10, 1 and 0.3% of the finished product, respectively). Turkey breast (5 g) was portioned into vacuum bags and inoculated with a four-strain cocktail of *B. cereus* spores to obtain ca. 2.5 log CFU/g. *B. cereus* growth data at isothermal temperatures (10, 15, 20, 25, 30, 35, 37, 39, 41, 43, 45, and 47 °C) were collected. The Baranyi model was used as a primary model to fit growth data; modified Ratkowsky model was fitted to the secondary model, and a tertiary dynamic model was developed using 4th-order Runge-Kutta method. The dynamic model was validated using a sinusoidal temperature profile, 10-40 °C for 48 h. Model fit parameters Root Square Mean Error (RMSE) and R^2 were used for the primary and secondary models and the accuracy and bias factors were used to evaluate the dynamic model.

Results: The mean RMSE and R^2 values for the primary model were 0.29 log CFU/g and 0.98, and for the secondary model were 0.05 and 0.92, respectively. The accuracy and bias factors for sinusoidal temperature profile were 1.10 and 1.12, respectively, which were within the acceptable range (0.75-1.25).

Significance: The developed model can be used to evaluate growth of *B. cereus* in turkey breast roast during processing (stabilization), storage and distribution.

P3-122 Hazard Analysis of Risk Factors By Microbial Risk Assessment from Farm to Table of *Bacillus cereus* for Lettuce

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Introduction: As the demand for lettuce continues to increase, the problem of microbial safety is also emerging. *Bacillus cereus* is widely present in the natural environment, which emphasizes the importance in the risk management of lettuce contaminated with *B. cereus* by analyzing all stages.

Purpose: The objectives of this study were to estimate the contamination of *B. cereus* from farm to table and to develop a simulation model for *B. cereus* in lettuce.

Methods: A growth predictive model was developed by utilizing the results of the examination of *B. cereus* growth pattern with respect to storage temperature in lettuce. Data of the hands of workers, bed soil, and soil that cause cross-contamination were collected through the survey of *B. cereus* contamination on lettuce in the production stage. Those data from the survey included temperature, time during transportation, and consumption data. A minimum infective dose (10^6 CFU) was used to develop the dose-response model. The risk of *B. cereus* due to ingestion of lettuce was simulated through the @RISK program.

Results: The initial contamination in seeds was estimated to be 0.9 Log CFU/g. The cumulative density determined by the simulation on the production stage showed that the number of estimated average *B. cereus* cells increased to 2.6 Log CFU/g. The average distribution of *B. cereus* contamination levels at the transportation stage were lowered from 2.6 Log CFU/g to 1.1 Log CFU/g. Average consumption of lettuce was 39.9 g, calculated by Exponential distribution [RiskExpon(39.839, RiskShift (0.0020030), RiskTruncate (0.03,503))] at 16.6% of frequency. As a result, we were able to conclude that the foodborne illness due to *B. cereus* occurred 3 times when the simulation model reiterated itself 10,000 times.

Significance: These results indicate that it is crucial for farmers to control the cross contamination level on the production stage.

P3-123 Strain Variability of Reduction Behaviors of *Campylobacter jejuni* Strains Under Isothermal Inactivation and the Bayesian Predictive Model of the Survival Kinetics

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Introduction: *Campylobacter jejuni* is one of the most widespread foodborne bacteria. Although the quantitative microbiological risk assessment (QMRA) has the potential to comprehend and control the risk of *C. jejuni* infections, the knowledge of the strain variability of thermal inactivation tolerances of *C. jejuni* is not enough to conduct the QMRA for *C. jejuni*. It is needed to reveal the magnitude of the strain variability *C. jejuni* thermal tolerance and to describe the reduction behavior with predictive models.

Purpose: The objective was to reveal the differences in survival kinetics of 19 strains of *C. jejuni* and to develop a predictive model describing the survival kinetics.

Methods: Nineteen strains of *C. jejuni* were used to account for the strain variability. Each suspension of the cultured *C. jejuni* of nineteen strains was heated under the isothermal condition at 55°C for 2.25, 4.5, 6.75, 9.0, and 11.25 minutes. The initial counts and survival counts were determined with the plate count method. And the survival kinetics were described with the Weibull model. For the fitting model, the Bayesian inference was used. The r-hut value was calculated for the confirmation of parameter estimation. A Kruskal-Wallis test was performed to verify significant differences in the parameter distributions describing each thermotolerance.

Results: The parameter distributions of Weibull model describing the reduction behaviors of 19 *C. jejuni* strains under heating at 55°C were successfully estimated using Bayesian inference because all r-hut values of parameter distributions were 1.0. The Kruskal-Wallis test significantly indicated that the parameters of the predictive model describing the thermotolerance for all strains of *C. jejuni* did not follow equal values (p-value<0.001).

Significance: There are significant strain differences in *C. jejuni* thermotolerance, and it is essential to develop predictive models that describe strain differences for accurate QMRA.

Poster

P3-124 Cross-Contamination of *Campylobacter jejuni* and Quantitative Risk Assessment: A Case Study of Chicken Processing Factory

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Introduction: *Campylobacter* species are common bacterial pathogens associated with human gastroenteritis worldwide. Poor handling during the processing of poultry products can often lead to cross-contamination with pathogenic bacteria especially during the processing, and eventually, give rise to the transmission of *Campylobacter* spp..

Purpose: This research aimed to evauate the different temperatures of cross-contamination via processing equipment of *Campylobacter jejuni* during the chicken products supply chain.

Methods: A case study company was carried out. Eight scenarios and four different temperatures were developed to estimate the transfer rates of *C. jejuni* with consideration to cross-contamination through processing equipment (gloves, knife, and cutting board) and thawing water. And an risk assessment for *Campylobacter jejuni* in studied company was carried out.

Results: The result showed that there is a considerable degree of cross-contamination during the processing of chicken. The transfer rates of *C. jejuni* between chicken meat and equipment or thawing water are highly variable, which cutting board and thawing water were the significant sources of cross-contamination. The highest transfer rate observed when transferred from inoculated chicken to cutting board at 25ºC (29.40%). An increase in temperature enhanced the transfer rate of *C. jejuni* from inoculated chicken to processing equipment and contaminated equipment to fresh chicken. The estimated exposure assessment showed the mean of chicken product exposure estimated value had 0.178 log CFU/6-kg package. Moreover, the mean risk of *C. jejuni* detected per package was 0.00814. This research concluded that the transfer rate from chicken to cutting board is the highest among all scenarios.

Significance: Investigation of the probability of bacterial transfer may provide the scientific basis for risk management and intervention strategies during the processing of raw chicken.

P3-125 Development of Mathematical Models to Describe the Kinetic Behavior of *Cronobacer Sakazakii* in Infant Snacks

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Introduction: As the new food market, such as food for customized and special dietary uses, is rapidly growing, it is necessary to investigate microbiological risk of foods, targeting infants and young children. Hence, it is necessary to investigate the kinetic behavior of *Chronobacter sakazakii* in the food.

Purpose: The purpose of this study was to develop mathematical models to describe the kinetic behavior of *C. sakazakii* in infant snacks.

Methods: A mixture of two *C. sakazakii* isolates was inoculated in a infant snack (puff type) at 4 log CFU/g. The inoculated samples were stored at 10°C, 15°C, 25°C, 30°C and 37°C for up to 25 days, and the bacterial cell counts were enumerated on chromogenic *enterobacter sakazakii* agar (CESA). The Baranyi model was fitted to the *C. sakazakii* cell counts to calculate the shoulder period (*SP*; day) and death rate (*DR*; log CFU/g/day). A square root model was then fitted to the kinetic parameters as a function of storage temperature. The model performance was evaluated with root mean square error (*RMSE*), A factor (A_f), and B factor (B_f).

Results: As storage temperature increased, the *SP* (1.0±0.6, 0.8±1.1, 0.1±0.1, 0.3±0.1, and 0.6±1.0 day) showed a tendency to shorten, and the *DR* (0.088±0.049, 0.083±0.018, 0.102±0.034, 0.400±0.184, and 0.592±0.130 log CFU/g/day) increased. The secondary models were appropriate to evaluate temperature effect on the *SP* and *DR* with 0.200 and 0.697 of R^2, respectively. *RMSE* (0.331), A_f (1.118), and B_f (0.981) indicated that the model performance was appropriate.

Significance: These results suggest that the developed models can be useful in predicting the fates of *C. sakazakii* in infant snack.

P3-126 Development of Predictive Models for *Cronobacter sakazakii* Growth in Powdered Porridge for the Elderly

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Introduction: As South Korea approaches to aging society, the demand for special-purpose foods targeting the elderly has increased and the related food market has been proliferating. Thus, the risk of *Cronobacter sakazakii* in elderly-friendly foods needs to be assessed in Korea.

Purpose: The objective of this study was to develop predictive models to describe the fates of *C. sakazakii* in powdered porridge, the elderly-friendly food.

Methods: A mixture of *C. sakazakii* strains (FDA 2370 and KCTC 2949) cultured in tryptic soy broth was harvested and freeze-dried, followed by mixing with porridge powder to minimize water activity change in the sample after inoculation. Subsequently, the inoculum was inoculated on the sample (10 g) at 4.0–5.0 LogCFU/g. The bacterial cell counts were enumerated on chromogenic enterobacter sakazakii agar during storage at 10°C, 15°C, 25°C, 30°C, and 37°C, and were fitted to the Baranyi model to calculate shoulder period (day) and death rate (LogCFU/g/day). The calculated parameters were analyzed with the square root model as a function of temperature. For the model validation, predicted data were compared with observed data to calculate *RMSE* (root mean square error), A factor (A_f), and B factor (B_f).

Results: As storage temperature increased, the shoulder period (5.9±1.2, 0.3±0.5, 0.0±0.0, 0.3±0.5, and 0.0±0.0 day) showed a tendency to shorten, and the death rate (0.053±0.022, 0.067±0.021, 0.131±0.084, 0.088±0.003, and 0.128±0.046 LogCFU/g/day) increased. The secondary models were appropriate to evaluate temperature effect on the death rate and shoulder period with 0.327 and 0.519 of R^2, respectively. For the model validation, *RMSE*, A_f, and B_f were 0.235, 1.042, and 0.999, respectively, showing the developed models were appropriate for growth prediction.

Significance: The models should be useful in describing the fates of *C. sakazakii* in the powdered porridge and showed the bacteria stay similar to initial cell counts at low temperatures, but cannot grow at high temperatures.

P3-127 Validation of a Competition and Dynamic Model for Shiga-Toxin Producing *Escherichia coli* (STEC) Growth in Raw Ground Pork during Temperature Abuse (10 to 40°C)

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Introduction: Recent epidemiological evidence suggests that pork products may contribute to STEC infections in humans. To predict the growth of STEC more realistically in pork, it is essential to evaluate competition models incorporating background microbiota.

Purpose: To validate the competition and dynamic model for the growth of various STEC serogroups (O157, non-O157, O91) in raw ground pork during temperature abuse.

Poster

Methods: Nalidixic acid (50 ppm) resistant (NAL+) STEC strains were inoculated (3-4 log CFU/g) into raw ground pork at two fat levels (ca. 5 and 25%). Five-gram pouches were submerged in water baths (10,15,20,25,30, and 40 °C) for 25 to 440 h. Cells were recovered on NAL+ MacConkey agar for STEC and 3M Aerobic plate count (APC) for background mesophilic microflora. TheJameson-No lag Buchanan model was fitted to the experimental data for each temperature separately. Maximum growth rates (μ_max) were modeled as a function of temperature using Cardinal parameter equation. The differential form of the Baranyi model with the Cardinal parameter equation was solved numerically using fourth order Runge-Kutta method in MicroRisk®. The dynamic model was validated using sinusoidal temperature profiles. Acceptable prediction zone (APZ) method were used to evaluate the model performance.

Results: The competition model was well fitted to the experimental data having 93% (1849/1981) residual errors within the desired APZ. Growth rates were not different between O157 and non-O157; however, serogroup O91 showed two to three times lower μ_max than other STEC at 10, 25, and 30 °C. The theoretical minimum and optimum growth temperature for all STEC groups ranged from 3.40 to 7.81 °C and 33 to 35 °C, respectively. The simulated dynamic model showed good prediction performance (pAPZ=0.98) to observed experimental data.

Significance: Industry and regulators can use the competitive and dynamic models to develop appropriate risk assessment and mitigation strategies to improve the microbiological safety of raw pork products.

P3-128 Quantitative Microbial Risk Assessment of *Salmonella* spp. and *L. monocytogenes* in Fresh Cabbage and Onion from Markets to Home

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Introduction: *Salmonella* spp. and *L. monocytogenes* are often detected in fresh produce, causing severe foodborne outbreaks worldwide.

Purpose: We conducted a quantitative microbial risk assessment of *Salmonella* spp. and *L. monocytogenes* in fresh cabbage and onion from markets to home.

Methods: The cabbages (n=180) and onions (n=174) from on and offline markets in Korea were analyzed for contamination levels of *Salmonella* spp. and *L. monocytogenes*. Predictive models of *Salmonella* spp. and *L. monocytogenes* in fresh cabbage and onion were developed as a function of time and temperature at 4 to 36°C . The daily consumption amount and intake rates of cabbage (26.38g, 0.32) and onion (33.28g, 0.59) were referred to the Korea National Health and Nutrition Examination Survey (n=8,063) and the report of Gallup Korea survey (n=1,201). The Beta-Poisson model of *Salmonella* spp. and the exponential model of *L. monocytogenes* were used as a dose-response model. Monte Carlo simulation analysis was run for the worst scenarios using @RISK.

Results: The initial contamination levels of *Salmonella* spp. and *L. monocytogenes* in cabbage (-3.91 log CFU/g) and onion (-3.89 log CFU/g) were increased at the markets (0~18.1°C) and during transportation (16.98~27.13°C). Growth of *Salmonella* spp. was faster than *L. monocytogenes* in both fresh cabbage and onion. The probability risk of illness caused by *Salmonella* spp. (1.65×10^{-3}) was higher than *L. monocytogenes* (6.31×10^{-8}) for the general population. The probability risk of listeriosis was increased to 100 times for the susceptible population. The risk of salmonellosis and listeriosis was mainly affected by market storage time.

Significance: Effective disinfection of fresh cabbage and onion during processing and time and temperature management at the on and offline markets should be emphasized for the microbial safety of fresh produce.

P3-129 Predictive Models for the Growth Kinetics of Uropathogenic *Escherichia coli* in *Sous-Vide* Processed Chicken Breast

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Introduction: Uropathogenic *Escherichia coli* (UPEC) is the most prevalent pathogen accounting for human urinary tract infection (UTI). Studies have shown that poultry meat, especially chicken, is a potential reservoir of UPEC, rendering a risk of foodborne UTI.

Purpose: The present study aimed to determine the growth kinetics of UPEC in ready-to-eat *sous-vide* processed chicken breasts using a one-step kinetic analysis method.

Methods: The phylogenetic type and virulence factor of UPEC clinical isolates were identified by polymerase chain reaction assay for related genes. A four-strain cocktail of UPEC (BCRC 10675, 15480, 15483, and 17383) was inoculated into *sous-vide* processed chicken breast at 3-4 log CFU/g and then stored at 4, 15, 20, 30, and 37 °C. The changes in the UPEC populations during storage were submitted to the USDA IPMP-Global Fit, a software for one-step kinetic analysis, to analyze the growth kinetics. The growth curves were fitted with six combinations of a primary model (Huang full growth or no lag phase model) and a secondary model (Huang square-root, Ratkowsky square-root, or cardinal parameters model). An additional inoculation study further externally validated the developed mathematical models as a function of temperature.

Results: The UPEC grew well in the chicken sample under 15,20,30, and 37 °C. The UPEC did not grow at 4 °C. Statistical indices showed that the combination of the no lag phase and the Huang square-root models was better than the others for fitting with the growth curves. An additional growth curve further validated this combination for predicting UPEC growth kinetics at 25 °C, showing that the RMSE, accuracy factor (A_f), and bias factor (B_f) were 0.96 (log CFU/g), 1.092, and 1.074, respectively, in an acceptable range.

Significance: The mathematical functions developed in this study can be applied to predicting the growth of UPEC in *sous-vide* chicken breast, which is useful for the exposure assessment or setting controlling measures of this pathogen.

P3-130 A Simulation of the Effect of Ground Beef Irradiation on Annual Nontyphoidal *Salmonella* and *Escherichia coli* O157:H7 Burden and Direct Healthcare Costs in the United States

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Introduction: Over 20% of *E. coli* O157:H7 illnesses and over 5% of *Salmonella* illnesses are estimated to be attributable to beef consumption in the United States. Irradiating ground beef is one potential method to reduce disease burden and healthcare costs.

Purpose: We simulated the effect of ground beef irradiation on illnesses, hospitalizations, deaths, and direct healthcare costs from ground beef-associated *E. coli* O157:H7 and *Salmonella* infections in the United States.

Methods: To estimate the fraction of illnesses, hospitalizations, deaths, and direct healthcare costs preventable by ground beef irradiation, we multiplied the disease burden attributable to ground beef; the estimated percentage of ground beef sold that is not currently irradiated; the percentage of unirradiated ground beef that would be irradiated; and the percentage reduction in risk of illness after irradiation. We multiplied this fraction by estimates of burden and direct healthcare costs to calculate the numbers or amounts averted. Model inputs were obtained from the literature and expert opinion. We used Monte Carlo simulation to incorporate uncertainty in inputs into model estimates. Simulation outcomes were summarized with means and 95% uncertainty intervals (UI).

Results: Irradiating 50% of the currently unirradiated ground beef supply would annually avert 3,285 (95% UI: 624–9,977) *E. coli* O157:H7 illnesses, 135 (95% UI: 24–397) hospitalizations, 2 (95% UI: 0–16) deaths, and $2,972,656 (95% UI: $254,708–$14,496,916) in direct healthcare costs. For *Salmonella*, 20,308

(95% UI: 9,858–38,903) illnesses, 400 (95% UI: 158–834) hospitalizations, 6 (95% UI: 0–18) deaths, and $7,318,632 (95% UI: $1,436,141–$26,439,493) in direct healthcare costs would be averted.

Significance: Increasing ground beef irradiation could reduce *E. coli* O157:H7 and *Salmonella* burden in the United States. Additional studies should assess whether targeted irradiation of higher-risk ground beef products could prevent similar numbers of illnesses with less total product irradiated.

P3-131 Modelling the UV-C Inactivation Kinetics and Determination of Fluences Required for Incremental Inactivation of Several Serotypes of Shiga-Toxin Producing *Escherichia coli* (STEC)

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Introduction: There is a disagreement in the literature about the UV-C dose required to inactivate STEC because of the failure to use standard methods and equipment, incorrect UV exposures calculated without considering the optical properties of the test liquid and finally, improper use of unit measures.

Purpose: Model the UV-C inactivation kinetics and determine the fluences required for the incremental inactivation of several serotypes of STEC suspended in clear buffer.

Methods: Five strains of each serotype; O157, O26, O45, O103, O111, O121, and O145 were individually suspended in PBS and treated with UV-C doses of 0, 2, 4, 6, 8 and 10 mJ/cm^2 using a collimated beam device emitting UV-C at 253.7 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 identified using the plate count method and plotted against UV-C dose and curve fitting using appropriate mathematical models was attempted. The UV-C dose required for incremental inactivation for each isolate was determined using linear and nonlinear regression.

Results: The D10 values of STEC strains are between 0.10 – 0.48 mJ/cm^2. Among the 35 isolates, a fluence of 10 mJ/cm^2 yields inactivation between 4.338 ± 0.215 and 7.712 ± 0.494 log CFU/mL. The best model for describing the log reduction of STEC versus UV-C fluence is the Weibull model with an average R-squared of 0.938.

Significance: A clear understanding of the UV-C dose response of different STEC serotypes in a clear buffer lays the foundation to determine the UV-C doses required to inactivate STEC in liquid foods and design successful UV based non-thermal pasteurization systems.

P3-132 Identifying the Best FIT Models Describing the Persistence of *Escherichia coli* O157:H7 in Fresh Vegetables Consumed in Salads

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Introduction: Whereas first order kinetics are commonly used to model decay of *Escherichia coli* O157:H7 (*Ec*O157:H7) in fresh produce, their simplicity may fall short of accounting for changes in decay dynamics due to population heterogeneity and other factors.

Purpose: This study aimed to document changes in the decay of *Ec*O157:H7 in fresh produce used as salads under different fate factors.

Methods: Datasets with at least four time points were extracted from literature sources documenting the fate of *Ec*O157:H7 in fresh vegetables used in salads that were identified as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Using linear modelling that fixed the y-intercept at zero, a suite of two one-parameter, nine two-parameter and six three-parameter models were fit to the log transformed microbial decay data. The best fit model was determined by evaluating the Bayesian Information Criterion (BIC) and the normalized root mean square error (RSME). T90 and T99 values, calculated from best fitting models, were as response variables in a variety of factor analysis that evaluated the fate factors.

Results: From the selected 66 articles, 368 datasets were extracted. Linear model identified 219 datasets with significant negative trend ($p<0.05$), of which 80% had at least one best fitting model identified. A two-parameter model provided the best fit to 80% of the datasets, whereas one-parameter model best fitted less than 25% of the datasets. Shredded lettuce was more likely to be chlorinated ($r=0.78$), whereas decontamination was less likely in primary production ($r=0.69$). Temperature, packaging and relative humidity were established as the most important factors influencing decay of *E. coli* O157 in fresh produce (AOR>0.51).

Significance: This study identified common nonlinear decay dynamics for EcO157:H7 on fresh produce used for salads, and provided parameter estimates for use in modelling under various conditions.

P3-133 Quantitative Analysis of the Effect of Weather and Time on the Survival of Generic *E. coli* on Oranges Following Foliar Spray Application

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Introduction: Agricultural water can be a source of bacterial, viral or protozoal pathogens which can contaminate fresh fruits and vegetables.

Purpose: We explored the survival of generic *E. coli* on oranges under different weather conditions to determine how conditions affect bacterial die-off.

Methods: Well water was mixed with fresh cow manure and adjusted to contain ca. 4 log CFU *E. coli*/ml for foliar spray application. Twenty-two field trials, on nine trees each, were conducted between October 2012 and May 2015, resulting in 198 experiments over 126 unique days. Three replicates of 10 fruits each were harvested from each tree, at each time point. Sampling occurred between 0 and 528 h after inoculation, for a total of 1,527 observations. The detection limit was -1 log MPN/orange. Data analysis was performed using the R statistical computing language version 4.0.3, the R Tidyverse libraries 1.3.0, and survival package 3.3-1. The bacteria counts were matched with the weather conditions of solar radiation, rainfall, air temperature, dew point temperature, relative humidity, and wind gusts, obtained from the Florida Automated Weather Network (FAWN) website.

Results: *E. coli* was detectable for a maximum of 6 h when the temperature was hotter than 26°C and the relative humidity did not exceed 90% (n=39). Using the Kaplan-Meier estimator, *E. coli* was detectable for at least 24 with median detectable time of 192 h, with a 95% confidence interval between 72 and 216 h, when the temperature surpassed 26°C and the relative humidity was at least 90% (n=92). The remaining experiments where the temperature was cooler than 26°C had a 95% confidence interval of 24 to 48 h (n=67).

Significance: Hot and dry conditions resulted in a rapid decline in the *E. coli* populations on oranges from poor quality agricultural water; survival of *E. coli* populations was favored by hot and humid conditions.

P3-134 Prediction of Time Temperature Control for Safety Status of Cottage Foods Based on Recipe Analysis

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Introduction: Cottage food laws favor food items that are classified as non-Time and Temperature Controlled for Safety (non-TCS). TCS food status is determined by pH and water activity (Aw).

Purpose: We investigated whether a food's TCS status can be predicted based on its ingredients.

Methods: Fifty-nine (59) recipes were obtained from two Texas cottage food cookbooks that contained laboratory analysis to determine TCS (n=20) or non-TCS (n=39) status. Each ingredient was separated into its components of fats, proteins, starch (i.e., complex sugars), simple sugars, salt, and water content, by mass. We fit linear regression and lasso regression models to predict water activity and pH. We fit logistic regression models using the ingre-

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dients' components as independent variables to predict whether a recipe was TCS or non-TCS. Recipe text mining was also performed. Data analysis was performed using the R statistical computing language version 4.2.2 and the R Tidyverse libraries version 1.3.1.

Results: Salt, sugar, and water content create a weak linear regression model of water activity (R^2 = 0.53). The best logistic model for predicting TCS had a training accuracy of 69.5% using the components of fats (p=0.05), sugars (p=0.02), and water (p=0.002). The specificity of the model (true negative rate) was 0.74, and the sensitivity (true positive rate) was 0.56. A pruned decision tree classifier split on starch and sugars, had a training accuracy of 0.8, specificity of 0.95, and sensitivity of 0.5. The application of heat (43 recipes) did not significantly impact the model, possibly due to the variability in baking times and temperatures, and text mining of recipes was unsuccessful.

Significance: The ability to predict TCS status based on baked food ingredients and recipes could aid cottage food producers and state health departments. The results here are promising but require further refinement.

P3-135 Comparison of Multiple Pathogen Growth Models Using Real World Transport Data for Leafy Greens

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Introduction: Many mathematical models are available for predicting pathogen growth in foods, including specialized models for foods like leafy greens. There has been a little systematic comparison of these multiple models to determine their suitability and differences in prediction.

Purpose: We compared multiple mathematical models of *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes* on leafy greens to determine prediction differences when real-world temperature data are used.

Methods: Temperature data were obtained from sixteen shipments that were monitored in 5-minute increments for transit temperature from June 2010 to May 2011. Relevant growth models were selected from the literature for *Salmonella* (n=6), *E. coli* O157:H7 (n=6), and *L. monocytogenes* (n=4). Data analysis was performed using the R statistical computing language version 4.2.2 and the specialized R Tidyverse libraries version 1.3.1.

Results: The *Salmonella* predictions from the Gibson and Puerta-Gomez models predict the least growth and were not statistically different from each other. The Mishra and Sant'Ana *Salmonella* models predict the most growth and were not significantly different from one another. Two other models (Veys and Koseki) were intermediary. The *Salmonella* model Kendall W effect size was 0.95, which indicates the risk ranking of shipments was consistent across *Salmonella* models. The Puerta-Gomez *E. coli* model predicted less growth than the other five models, and the Buchanan, McKellar, and Veys models predict more growth than the other models. The *E. coli* Kendall W effect size was 0.95, which indicates the choice of *E. coli* model does not appreciably alter the risk ranking of shipments. The *L. monocytogenes* models from Mishra Sant'Ana, Koseki, and Buchanan are also significantly different, with a Kendall W effect size of 0.97.

Significance: While model predictions were different, high Kendall W effect sizes indicate that model choice has minimal impact on risk management once a risk tolerance is set.

P3-136 Modeling the Combination Effects of Temperature, pH, Water Activity, Nitrite, and Organic Acids on the Growth of *Listeria monocytogenes* in Processed Meat Products

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Introduction: Delicatessen meats are reported to be the leading vehicle of foodborne *listeriosis* outbreaks with a high fatality. Predictive models are valuable tools to assess and manage public health risks.

Purpose: The objective of this study was to develop and validate a global model based on food matrices' literature data for predicting the growth of *Listeria monocytogenes* in processed meat products.

Methods: The model considers the effect of seven environmental parameters including temperature, pH, water activity, nitrite, acetate, lactate, and propionate with and without interaction effects. Experimental growth curves of *Listeria* in food matrices (beef, pork, and poultry) were collected from ComBase, or modeled from research publications, and analyzed, after which a total of 414 growth curves were retained. The four-parameter logistic model with delay was used as the primary model to estimate the growth parameters (N_o, $N_{max,}$ tlag, μ_{max}) for each curve.

Results: The growth rate data were divided into two sets, about 355 data were used to build the model, and 135 food matrix datasets for validation. A gamma concept-based bespoke model was developed and performance was evaluated using bias factor (Bf), accuracy factor (Af), and acceptable simulation zone (ASZ) of ± 0.5 log cfu/unit. The developed global model had an acceptable Bf of 1.02, Af of 1.17, and ASZ score was more than 72%. Therefore, the gamma secondary model developed using food matrix data provided a valid prediction for the growth of *Listeria* in processed meat products.

Significance: This study is the first to develop a model that considers nitrite, propionate, acetate, and lactate as combined inhibitors of *Listeria*, along with temperature, pH, and water activity. The results of our study provide significant insights into the need for the development and validation of predictive models in real food matrices rather than laboratory media alone. The model may be useful in timely decision-making and quantitative risk assessment in RTE-cooked meat products.

P3-137 Estimation of *Listeria monocytogenes* Levels within Apple Production Environments Utilizing Reverse Quantitative Microbial Risk Assessment

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Developing Scientist Entrant

Introduction: A 2014 outbreak from consumption of caramel apples contaminated with *Listeria monocytogenes* highlights a current gap with modern food microbial detection technology limits and *L. monocytogenes* contamination levels.

Purpose*:* As the 2014 outbreak proves, *Listeria monocytogenes* prevalence becoming ubiquitous within produce environments accentuates the need to investigate concentration levels of the pathogen within the handling facilities.

Methods: This study uses Quantitative Microbial Risk Assessment (QMRA) to analyze a Listeriosis outbreak from 2014 associated with contaminated caramel apples. QMRA is a modeling framework that commonly uses environmental sampling data to characterize pathogen fate, transport, and the likelihood of adverse health effects. However, in reverse, epidemiological study results from the outbreak can be used to estimate pathogenic levels. To capture human variability, two models were constructed (average-adult & pregnant women-stillbirths) with four consumer handling scenarios based on storage temperature (7 °C, 25 °C) and storage duration (1 day, 1 week). 1000 iterations of Monte Carlo simulations were conducted to supply a resultant distribution of *Listeria monocytogenes* estimates to better minimize uncertainty associated with each model.

Results: The average adult model's scenarios of room temperature short & extended and cold storage short & extended estimated mean values of 0.147,0.0713, 0.198, and 0.0754 Colony Forming Units per gram (CFU/g), respectively, in comparison to the stillbirth model's results of the scenarios that provided mean values of 1.24, 0.601, 1.67, and 0.635 CFU/g, respectively, demonstrate a very low-level prevalence of the pathogen within these environments. Spearman rho correlation estimates determined the most uncertainty for both average adult and stillbirths lies in the growth parameter for 75% of the consumer handling practice scenarios except short term cold storage.

Significance*:* The findings from the reverse QMRA will be used to inform policymakers and food science technology about the limitations of current detection methods utilized for low-concentration pathogens, such as *L. monocytogenes*.

Poster

P3-138 Modeling the Colonial Growth Dynamics of *Listeria monocytogenes* Single Cells after Exposure to Sublethal Food Processing-Related Stresses

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❖ ***Developing Scientist Entrant***

Introduction: Exposure of *Listeria monocytogenes* to sublethal stresses may induce different physiological states i.e., viable, sublethally injured or dormancy, that present variable resuscitation capacity.

Purpose: (i) To monitor the real-time recovery of *L. monocytogenes* single-cells after exposure to different sublethal stresses; (ii) to investigate the heterogeneity in the colonial growth dynamics of stressed single-cells; and (iii) to detect non-dividing fractions.

Methods: Exposure to acetic acid-AA (pH 3.0; for 5h), hydrochloric acid-HCl (pH 3.0-2.5; for 5h) and peracetic acid-PAA (10ppm; for 3h) at 20°C were used to induce different physiological states in *L. monocytogenes*. After stress exposure, colonial growth of single-cells was monitored, on Tryptic Soy Agar supplemented with 0.6% Yeast Extract by time-lapse microscopy, at 37°C. Images were acquired every 5min and were analyzed using BaSCA pipeline. The obtained colonial growth curves were fitted to the model of Baranyi and Roberts for the estimation of lag time-λ and maximum specific growth rate-μmax. Data analysis and modelling were done in R.

Results: Growth of 113 untreated single-cells was monitored. Eleven (9.73%) were non-dividing. After treatment with AA, 12 (24%) out of 50 single-cells found as non-dividing. The average λ value and μmax of growing cells was 1.68h (SD=0.53h, %CV=32.0%) and 14.61h^{-1} (SD=49.93h, %CV=341.69%), respectively, compared to 1.57h (SD=0.57h, %CV=36.32%) and 3.46h^{-1} (SD=24.74h, %CV=714.24%) of the control. The behavior of 61, 59, 58 single-cells treated with HCl at pH 3.0, 2.7 and 2.5, respectively, was investigated. The average λ value of HCl-treated cells ranged from 1.11h (pH 2.7) to 1.57h (pH 3.0). Exposure to PAA 10ppm resulted in 51 non-dividing cells out of 81 (62.96%). Their λ value ranged from 0.47h to 2.84h and the average μmax was 31.53h^{-1} (SD=71.71h, %CV=227.43%).

Significance: Assessing the variability of the colonial growth dynamics after stress exposure offers quantitative insights on the impact of stress on residual risk associated with survivors.

P3-139 Validating Agent-Based Model (ABM) That Predicts *Listeria* spp. Prevalence on Environmental Surfaces in a Retail Store

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Introduction: *Listeria* spp. are index microorganisms for *Listeria monocytogenes*, which may be transferred via various routes in a retail environment.

Purpose: The goal of this study is to provide better tools to assess and optimize *Listeria* interventions in retail. We specifically aimed to improve and validate an ABM for predicting *Listeria* transmission in retail produce sections and identify key data needs for future models.

Methods: A preliminary ABM was substantially improved by (i) implementing new routes of *Listeria* introduction, (ii) including more agents, and (iii) utilizing material-specific transfer coefficients for microbial cross contamination. *L. monocytogenes* prevalence data from different retail surfaces were collected from a previous longitudinal study and were used for validation. Prevalence data for specific agents were generated at four time points over a simulated month of a retail operation over 1000 iterations. Model predictions were validated for all agents included in both the model and the validation data. Sensitivity analysis was performed using Partial Rank Correlation Coefficient to identify the key parameters that affect mean prevalence.

Results: Prevalence data for 41 out of 55 agents were within 95% confidence interval of the validation data, while simulated *Listeria* prevalence was higher for 10 agents representing sites classified as consumer scale (8 agents) and dry display shelf (2 agents). Sensitivity analysis showed that (i) transfer coefficient for stainless steel to employee's hand, (ii) number of maintenance events, and (iii) transfer coefficient for cardboard to stainless steel were the top three parameters that were significantly ($p < 0.05$) associated with the mean *Listeria* prevalence across all agents, which suggests better characterization of these parameters is necessary.

Significance: The validated model can be used as a tool to predict *Listeria* transmission in retail stores and will be further developed to assess different *Listeria* control strategies.

P3-140 Controlling Persistent *Listeria* in Food Retail: Adaptation of Analytical Approaches for Risk Assessment, Root Cause Analysis, and Intervention

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Introduction: *Listeria monocytogenes* is a major foodborne pathogen associated with the food processing environment. Adapting various analytical approaches to conduct risk assessment and root cause analysis of persistent Listeria will improve the capability of global food retail chains to develop a proactive intervention plan.

Purpose: Limited studies have examined analytical methods to conduct root cause analyses and interventions for Listeria in food retail. The purpose of this study is to utilize literature review to identify the most important analytical approaches and compile them in a customized roadmap based on different sizes of food retail establishment.

Methods: A protocol was followed to conduct search in NCBI-PubMed database and google to collect studies and guidelines related to Listeria monocytogenes and root cause analysis. Several terms were used in the search including "persistent Listeria", "Listeria monocytogenes Biofilms", and "root cause analysis".

The Listeria relevant studies were sorted by inclusion criteria such as conducted in food facilities, *L. monocytogenes*, persistent Listeria, harborage sites, and microbiological sampling or simulation. The root cause analysis studies focused on quality assurance, and machine learning tools. The results and tools were drawn from all relevant studies and combined into a roadmap tailored to food retail.

Results: A total of 25 studies were examined, including U.S. and international studies and guidelines. Six analytical approaches for root cause analysis and interventions were identified: Agent-Based Model, cause-and-effect diagram, interrelationship diagram, 5-whys, machine learning, and quantitative risk assessment. A roadmap was developed to adapt these tools to the food retail. The roadmap has three stages: (1) risk assessment and focus plan, (2) source and root cause analysis, and (3) interventions and control.

Significance: The identified tools and the roadmap provide a simplified and customizable insight into the analytical approaches and enhance the intervention to control persistent Listeria.

P3-141 Development and Validation of a Predictive Growth Model for *Listeria monocytogenes* in Egg Yolk

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Introduction: Liquid egg products undergo pasteurization, however, post-pasteurization contamination with *Listeria monocytogenes* during transportation or storage may cause the product to become unsafe for consumption.

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Purpose: To develop a dynamic predictive growth model for *Listeria monocytogenes* in egg yolk and validate the model with plain, salted, and sugared egg yolk.

Methods: A six-strain *L. monocytogenes* cocktail was inoculated into plain liquid egg yolk. Growth data was collected at isothermal profiles of 0, 2, 5, 10, 15, 20, 25, 30, 35, 40, 42.5 and 45 °C. The Baranyi model was used to describe *L. monocytogenes* growth kinetics at each isothermal temperature. An estimated maximum specific growth rate for each temperature was fitted into the Ratkowsky square root model. The fourth order Runge-Kutta method was used to predict bacterial growth under non-isothermal conditions. The predictive model was validated for *L. monocytogenes* growth in plain, 10% sugared, and 10% salted egg yolk using three dynamic temperature profiles.

Results: The maximum specific growth rate increased from 0.0052 to 0.4397 log CFU/h at 0 and 35 °C, respectively. The theoretical minimum and maximum temperatures of growth obtained from secondary modeling were -0.32 and 45.35 °C, respectively. The model underpredicted *L. monocytogenes* growth in plain egg yolk when the average prior physiological stage (h_o) was 0.01, however, the prediction errors were within the acceptable range. For sugared egg yolk, the model overpredicted *L. monocytogenes* growth when h_o = 0.01. Salted egg yolk showed either no growth or a decline in *L. monocytogenes* population.

Significance: The dynamic model can be used to help processors determine microbiological safety of egg products in cases of temperature abuse.

P3-142 Quantitative Modeling of *Salmonella* spp. Survival in Soy Sauce-Based Products

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❖ ***Developing Scientist Entrant***

Introduction: Soy sauce-based products are generally considered in the acidified food category, for which validation study of 5-log reduction by acidification or any pasteurization process must be conducted. *Salmonella* spp. could be a potential pathogen of concern in this type of products due to the frequent detection of *Salmonella* in soybean and the emergence of strains with elevated acid tolerance

Purpose: To develop quantitative primary and secondary model of *Salmonella* spp. survival in soy sauce-based products under various pH, soluble solid, NaCl, and soy sauce compositions.

Methods: A five-strain cocktail of *Salmonella* spp. were acid adapted and inoculated to 30 combinations of soy sauce formula generated using central composite design (CCD), with the consideration of four independent variables [pH, soluble solid, salt content, and percent composition of soy sauce]. The primary model of *Salmonella* survival was developed on based on the microbial enumeration at 16 timepoints in a period of 28 days. Kinetic parameters from the best-fit primary model were modeled in secondary polynomial regression as a function of the four variables, following a stepwise selection procedure.

Results: Weibull model [δ (h); *p*] was determined as the best-fit primary model to quantify the survival of *Salmonella* spp. in soy sauce-based products. The logarithmically transformed scale and shape parameters were modelled in the secondary model, resulting in an acceptable fitting for both internal and external validations. Results indicated that increasing percent of soy sauce, salt, soluble solid and decreasing pH can accelerate the inactivation of *Salmonella* spp.

Significance: The integration of primary and secondary models from the study could be used to determine an adequate holding time required to achieve necessary *Salmonella* spp. inactivation in acidified cold-fill-hold soy sauce-based products.

P3-143 Modeling and Optimum Experimental Design of *Salmonella* Inactivation in Inoculated Wheat Flour

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❖ ***Developing Scientist Entrant***

Introduction: Previous research involving consumer-based thermal heat treatments of raw flour is limited in scope. Generation of an inactivation model and optimum experimental design parameters will aid further investigations of the proposed consumer-based thermal treatments.

Purpose: The objective was to estimate modeling parameters for the inactivation of *Salmonella* enteritidis PT30 using data gathered from pilot-scale experiments.

Methods: Multiple parameter estimation methods were conducted for comparison. Data were collected from pilot-scale thermal inactivation study of *Salmonella* in wheat flour. The primary log-linear model was combined with the Bigelow secondary model to estimate inactivation within non-isothermal treatment datasets at 121 and 177°C. The target parameters were $D_{90°C}$ (min), z (°C), and log(N_0) (log CFU/g). MATLAB was used to estimate parameter values through scaled sensitivity coefficients (SSC), ordinary least squares estimation (OLS), sequential estimation, and bootstrapping methods. Plots of residuals, SSC, confidence bands, and prediction lines were created at relevant estimation step.

Results: The resulting parameter values for both OLS and sequential estimation were $D_{90°C}$=4.53 min, z=69.3°C, and log(N_0)=8.51 log CFU/g. Bootstrap parameter values were $D_{90°C}$=4.76 min, z=65.0°C, and log(N_0)=8.44 log CFU/g. Parameter values were similar in scale and converged after the implementation of each estimation method. SSC plots of the parameters indicated an ability to most accurately predict log(N_0) and to least accurately estimate z, with corresponding levels of lower and higher relative errors.

Significance: An iterative, multiple-estimation method approach to modeling *Salmonella* inactivation mechanisms within flour can optimize parameter estimates, provide insight to the nature of the model parameters and the errors they may contain, and inform the course of further experimentation. The generated model may have applications with other consumer-based thermal treatments in low-moisture foods.

P3-144 Effect of Different Heating Processes on the Survival and Inactivation of *Salmonella* Seftenberg in a Cell-Cultivated Salmon Matrix

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Introduction: The advent of Cellular Agriculture has led to the development of a variety of novel foods. Among these are Cell-Cultivated foods, foods derived from the cell cultures of animals. Cell-cultivated salmon, being a novel food, requires safety assessments of the product and processes associated with its production.

Purpose: The objective of this study was evaluating the survival and inactivation parameters of *Salmonella* spp. including a heat resistant *Salmonella* Seftenberg, after subjection to a variety of heating processes that can potentially be used in cell-cultivated production settings of a Salmon matrix.

Methods: Cell-cultivated Salmon was provided by Wildtype, a San Francisco-based producer of cultivated seafood products. A 5-strain cocktail of Salmonella spp., including *S.* Seftenberg, *S.* Typhimurium, *S.* Enteritidis, *S.* Infantis, and *S.* Newport was inoculated in 100µl aliquots to 5 g samples of Cell-cultivated Salmon. These samples were sealed in vacuum bags and rested at 20°C for a 30-minute attachment time before immersing in a water bath simulating different heating processes at 57.5°C, 60°C, and 62.5°C. Samples were collected at different intervals during heating to develop inactivation curves. After sample collection, bags were immersed in an ice bath to arrest the heating effect and enumerated by direct plating on TSA agar using microdilution methods, drop plate, and spread plating. Linear Regression Statistics were performed in R.

Results: Salmonella was inactivated at the three temperatures evaluated in this study. D values in minutes for the *Salmonella* spp. cocktail were found to be 2.93±0.19, 0.74±0.06, and 0.38±0.02 for 57.5°C, 60°C, and 62.5°C, respectively with a $P<0.05$.

Significance: These data shows that Salmonella spp., including heat resistant strains can be inactivated in cell-cultivated salmon matrix when subjected to mild processing temperatures during cell-cultivated seafood production.

P3-145 Continuous Improvement of the Canadian Food Inspection Agency's Establishment-Based Risk Assessment Model 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 model (ERA-Hatchery model) to evaluate hatcheries based on the food safety risk related to *Salmonella* spp. they represent to Canadian consumers' health and 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.

Methods: 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 (2.4× reduction) and layer breeders (4.2× reduction), both showing the lowest Canadian hatchery production volume for the poultry meat and table-egg production chains, respectively. In counterpart, results showed an increase in the RR associated with turkey and layers/layer breeders for the poultry meat production chain (1.3× and 1.7× increase, respectively). 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 the ERA-Hatchery model, enabling a more meaningful distribution of the food safety burden across hatcheries and thus improving the risk-informed allocation of inspection resources.

P3-146 Monte Carlo Simulation of *Salmonella* Cross-Contamination in Dairy Powder Processing Environments

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Introduction: *Salmonella* has increasingly been implicated in outbreaks of low moisture foods since it can survive in low moisture processing environments, cross-contaminate low moisture foods, and then persist in contaminated food products.

Purpose: The purpose of this study was to model how a *Salmonella* contaminated food contact surface can cross-contaminate dry dairy powders over the course of a production campaign using Monte Carlo simulations.

Methods: Stainless steel coupons (L*W*H: 3.5*2.4*0.48 cm^3) were spot-inoculated with *Salmonella Enteritidis* PT30 (10^4 – 10^6 CFU) and then exposed to milk powder. Transfer of *Salmonella* into milk powder was enumerated and used to calculate bacterial transfer rates. Transfer rates were fitted to a log-normal distribution in R using the fitdistrplus package v1.1.8. A Monte Carlo simulation was designed in R that models the cross-contamination of *Salmonella* from a single contaminated surface (10^2 CFU, 10^4 CFU, or 10^6 CFU) into milk powder during a production run. Production runs (n=1,000) producing 1,200 units of milk powder product were simulated.

Results: A surface contaminated with 10^2 CFU of *Salmonella* resulted in an average of 5% (P5: 4%, P95: 6%) of milk powder product units being contaminated with mean 1.5 (P5: 1, P95: 2) CFU during a simulated production run. It took an average of 497 (P5: 324, P95: 717) units being processed before all CFU was removed from the contaminated surface. When the starting surface contamination was 10^6 CFU, the average percent of contaminated units increased to 73% (P5: 66%, P95: 80%) and the mean contamination level of product increased to 1145 (P5: 1047, P95: 1263) CFU. A full production run of 1200 units was not enough to fully remove the starting contamination (10^6 CFU).

Significance: Monte Carlo simulations can model the cross-contamination of *Salmonella* in low moisture food products. This can inform improvements in sanitation techniques used in low moisture facilities.

P3-147 A Dynamic Predictive Model for the Growth of *Staphylococcus aureus* in Raw Bacon and Potential Toxin Production

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Introduction: Growth of *S. aureus* in meat and poultry products can result in production of heat stable enterotoxin in the food and subsequent heat treatments may not alleviate the risk of foodborne illness.

Purpose: Develop a dynamic growth model for *Staphylococcus aureus* in raw bacon to predict potential growth and its toxin production during cooking process deviation.

Methods: Fresh pork belly meat (lean) was ground and mixed with a marinade containing water, salt, phosphate, sodium erythorbate, sodium nitrite (10%, 1%, 0.3%, 550 ppm and 100 ppm, respectively, of the finished product). Ground bacon (5 g) was inoculated with a five-strain cocktail of *S. aureus* to obtain 2.5 log CFU/g. Baranyi model was used to fit *S. aureus* growth at isothermal temperatures between 15 and 47.5ºC, modified Ratkowsky model was fitted to the secondary model and a tertiary dynamic model was developed using 4th-order Runge-Kutta method. The dynamic model was validated using a sinusoidal temperature profile, 10-40 °C for 48 h. Model fit parameters Root Square Mean Error (RMSE) and R^2 were used for the primary and secondary models and the accuracy and bias factors were used to evaluate the dynamic model.

Results: S. aureus did not grow at ≤10ºC and >47.5ºC in raw bacon. The theoretical T_{min} and T_{max} values were estimated as 5.3 and 46.2ºC, respectively. The mean RMSE and R^2 values for the primary model were 0.34 log CFU/g and 0.97, respectively. The accuracy and bias factors for sinusoidal temperature profile were 1.08 and 1.04, respectively, which were within the acceptable range (0.75-1.25). *S. aureus* enterotoxin (SE) was produced at *S. aureus* populations of ≥6.26 log CFU/g in raw bacon meat.

Significance: This model can be successfully used for predicting *S. aureus* growth and potential SE production in raw bacon during heating process deviations.

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P3-148 Predictive Model for Growth of *Staphylococcus aureus* at Temperatures Applicable to Cooling of Cooked Foods

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Introduction: *Staphylococcus aureus* continues to be a pathogen of concern to the food industry. Staphylococcal food poisoning outbreaks may occur due to holding of foods at unrefrigerated temperature for several hours prior to cooking or due to post process contamination and subsequent holding of foods at a warm temperature for an extended period to allow multiplication of the pathogen with concurrent production of enterotoxin.

Purpose: To develop a dynamic predictive model for *S. aureus* in Tryptic Soy Broth (TSB) over the growth temperature range of 10 to 45°C.

Methods: S. aureus growth was quantified in 50 ml flasks of TSB inoculated with ca. 2 log CFU/ml, and then incubated at isothermal conditions between 7 to 48.9 °C. Samples were removed periodically and plated on Tryptic Soy Agar (TSA). The plates were incubated for 24 h at 37°C. Data were fitted into primary Baranyi growth model that estimated the four parameters, physiological parameter (h0), initial and maximum population density and maximum specific growth rate (μ_{max}). Thereafter, modified Ratkowsky secondary model was fitted to the μ_{max} and lag time as a function of temperature.

Results: Both primary and secondary models fitted the growth data well as depicted by the goodness of fit measures (high coefficient of determination (R^2); low root mean square error, RMSE). The average value of h_0 was 3.398, averaged across all the growth temperatures from 7 to 48.9°C . Estimated maximum and minimum growth temperatures were 47.3 and 5.7°C , respectively.

Significance: Dynamic predictive model will assist regulatory agencies and food service establishments to estimate potential *S. aureus* behavior in food products stored at improper temperatures as well as to determine compliance with the regulatory performance standards.

P3-149 Microbial Risk Assessment of Norovirus and Hepatitis A Virus by Fresh Strawberry Consumption

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Introduction: The foodborne illnesses by norovirus (NoV) and hepatitis A virus (HAV) are frequently reported, and fresh strawberries have been related to viral foodborne outbreaks.

Purpose: The objective of this study was to estimate the risk of NoV and HAV foodborne illnesses by the consumption of fresh strawberries in Korea.

Methods: Fresh strawberries (*n*=247) were collected from markets and analyzed to detect NoV and HAV. To describe the fates of NoV and HAV in strawberry samples, predictive models were developed with the plaque counts of the virus collected at 4-25°C. The time and temperature for market display, transportation and home storage were collected, and the consumption data were surveyed. Dose-response models were searched through previous studies. With these data, the probabilities of NoV and HAV foodborne illnesses were estimated through the Monte Carlo.

Results: Of 247 samples, NoV was not detected, butHAV was detected in 1 sample in the samples, and the initial contamination levels were estimated to be –7.0 and –6.6 Log PFU/g, respectively. The predictive models showed that levels of NoV and HAV decreased as temperature increased. Market display time and temperature were fitted with the Uniform distribution (0, 24) and the Uniform distribution (22, 24), respectively. Transportation temperature and time were fitted, using the Uniform distribution (0.325,1.643) and the Uniform distribution (10,18,25), respectively. Home temperature and time were fitted with the LogLogistic distribution (-29.283,33.227,26.666,RiskTruncate(-5,20)) and the Uniform distribution(0,96) , respectively. Average consumption amount was 91.9 g. Dose-response models were Risk=1-(1+ηCV)$^{-r}$ (η, 2.55×10^{-3}; CV, dose; r, 0.086) for NoV, and Risk=1-(1+dose/*β*)-*α* (*α*, 0.373; *β*, 186.4) for HAV. The simulations with these data showed the probabilities of the foodborne illness were 3.71×10^{-11} and 9.81×10^{-10}/person/day for NoV and for HAV, respectively.

Significance: This result suggests that the risk of NoV and HAV foodborne illnesses by fresh strawberry consumption can be considered low in Korea.

P3-150 Determination of Critical Control Points in Green Pepper Production by Microbial Risk Assessment

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Introduction: There are many points of control microbial risk, but it may not be efficient because there are too many steps to be controlled.

Purpose: The objective of this study was to determine critical control points to improve control points for *Bacillus cereus* in green pepper by the microbial risk.

Methods: The cause of cross-contamination in the stage of harvesting peppers was identified and applied to the risk assessment scenario. The *B. cereus* of cross-contamination sources was identified. The *B. cereus* cell counts transferred to green peppers by cross-contamination were estimated with the cross-contamination rate. To describe the fates of *B. cereus* in green pepper, predictive models were developed at 7 °C, 15 °C, 25°C and 37°C. The time and temperature for the farm and transfer to consumers were collected, and the consumption data were surveyed. The simulation models were prepared for online and offline markets, and the simulations were performed by Monte Carlo simulation in @RISK.

Results: The predictive models showed that levels of *B. cereus* decreased as temperature increased from 15°C to 37°C. The *B. cereus* cell counts in peppers increased because of gloves at farms, but the cell counts during storage at due to refrigeration temperature before the shipment. And the *B. cereus* cell counts decreased to –8.0 Log CFU/g during the storage and washing at home. The average consumption amount of green pepper was 19.9 g per person at 8.2% of consumption rates. The simulation showed that harvest gloves and packaging gloves had a positive correlation for the risk, but low-temperature storage after the harvest had a negative correlation for the risk.

Significance: This result suggests that gloves used for harvest and packaging should be critical points in green pepper production, and it may improve the efficiency to control *B. cereus.*

P3-151 Smoking Causes Propionic Acid in Production in Salmon

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Introduction: Propionic acid, benzoic acid, and sorbic acid are used widely as preservatives in the food industry. These preservatives can be added to food only up to the concentration permitted in the regulation. However, preservatives are often detected in food, even though the preservatives are not added to food. Thus, the evaluation is necessary for the production of natural preservatives in food.

Purpose: The purpose of this study was to investigate the production of propionic acid, benzoic acid, and sorbic acid during the manufacturing process of smoked salmon.

Methods: Raw salmon samples are divided into samples of fresh and non-fresh (stored at room temperature for 6 h), and raw salmon samples were salted, washed, smoked, and cooled. This process was repeated three times. Smoked salmons were subjected to the analysis of the preservatives. The concentrations of propionic acid, benzoic acid, and sorbic acid were determined by HPLC and GC during the manufacturing process.

Results: In the smoked salmon processing stage, propionic acid was not detected after raw materials, salting and washing. Even though no propionic acid was added in the smoking process, 2.6 – 11.4 ppm of propionic acid were detected in the samples after smoking and after cooling, regardless of the freshness of raw salmon. Benzoic acid and sorbic acid were not detected in the samples during the manufacturing process.

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Significance: This result indicates that the smoking process may cause propionic acid production in salmon, and the freshness of raw salmon may not affect the production.

P3-152 Development of Cilantro Pre-Harvest and Harvest Model for *Cyclospora cayetanensis* Testing

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Introduction: Optimum food safety test and reject (sampling) plans in the produce industry are limited by the inconsistent requirement for product sampling. Currently, there is limited information about *C. cayentensis* testing performance in cilantro.

Purpose: This study develops a model that utilize current literature based on the FDA recommended sampling methods for *C. cayentensis* testing in cilantro to assess the sampling performance in a harvest season.

Methods: A simulation was built in Python to represent *C. cayentensis* dynamics in a harvest season of cilantro (45 days) assuming contaminated water was used for irrigation (from 0.6oocyst/L, up to 20oocyst/L) in a 22,000 lbs field. Cyclospora testing performance for water and fresh produce sampling were based on FDA recommendations for parasitic animals in foods and other available literature. Empirical data obtained from literature was used to create a logistics regression fit to obtain the probability of detection according to oocysts present in a sample.

Results: For water testing, contamination simulation showed that above 2 oocyst/L the probability of detection is 100% for all number of samples. In contamination under 1 oocyst/L, the number of samples matter. In low contamination, 1 sample has low probability, but after 2 oocyst/L it has reliable detection. For product testing, results showed that above 0.5 oocyst/gram the probability of detection increases to 100%, even 1 test will detect contamination. For lower levels of contamination, the number of testing samples will affect the probability of detection. Under 0.5 oocyst/gram, the number of samples matter.

Significance: This study asses the *C. cayetanensis* probability of detection of current sampling plans in available literature based on different contamination levels in a water or produce sample.

P3-153 Assessment and Translation of *in Vitro* weak Organic Acid Resistance Models of Filamentous Fungi in Bakery Applications

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Introduction: Propionic acid (PA) and acetic acid (AA) are weak organic acids used in food industry to increase mold-free shelf life of bakery products.

Purpose: This study aims to correlate *in vitro* experiments to in-product shelf life. This will accelerate product development and reduce resources used in traditional shelf-life testing with various weak acid compositions.

Methods: Several filamentous fungal species (*Penicillium roqueforti*, *Penicillium commune, Aspergillus niger* & *Cladosporium cladosporioides*) were inoculated on potato dextrose (PD) media and on white bread containing PA (0 - 45 mM) and AA (0 – 250 mM) at different pH levels. After inoculation, the cultures were incubated at 25 °C and fungal growth was monitored over time. A modified Gompertz equation was employed to determine fungal lag times (λ; i.e. time to growth). In addition, dose response curves were generated for PA and AA in both media and bread.

Results: Fitting the Gompertz equation did not result in significant differences between lagtimes on bread (λ_{bread}=4.48±0.496 SE) or on PD media (λ_{PD} = 4.25±0.288 SE). Analyzing multiple combinations of CP or AA in both media types showed that the growth profile was similar for both media types. A secondary model (Hill equation) shows that the PD media growth curves have the potential to be a substitute for in-food experiments.

Significance: Assessment of fungal growth in food matrices is not straightforward. This study demonstrates the potential of *in vitro* experiments can lower the number of challenge studies for developing new products against filamentous fungi.

P3-154 Statistical Framework for Surrogate-Based Validations of Preventive Controls and Optimal Data Collection

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Introduction: Non-pathogenic surrogate organisms are a popular tool for processors validating preventive controls because of the availability of surrogate-based validation guidelines. However, detailed experimental designs and statistical analyses are missing from many such guidelines.

Purpose: The goal was to identify optimal data collection and analysis practices for ideal and non-ideal surrogates for use in preventive control validations.

Methods: Using Matlab and Monte Carlo methods (1000 iterations), simulated datasets were generated mirroring common validation practices. Datasets were evaluated using an acceptance criterion of ≥95% of predicted samples exceeding the target lethality (≥95% predicted process performance), from which the aggregate was evaluated using an acceptance criterion of ≥80% statistical power. Using this framework, the impact of the number of positive controls (n=3-10), independent replications (n=3-6), samples (n=5-18), and true process performance (90 – 99% of samples exceeding target lethality) on the statistical power of the experimental designs were examined. Common considerations for surrogate validations were also included, such as non-ideal surrogate characteristics and samples below the limit-of-detection (LOD).

Results: The true process performance of preventive controls was the strongest indicator for statistical power; no experimental designs tested resulted in ≥80% statistical power for process performance near the acceptance criteria (94-96%). Otherwise, increasing data collection improved statistical power; optimal data collection schemes were identified. Substituting samples below the LOD with the LOD improved the likelihood of achieving the >95% acceptance criterion; however, this was a negative feature for under-achieving processes (<95% process performance). Surrogate-based results were conservative representations of pathogen-based results only if surrogate resistance and variability both exceeded the pathogen's.

Significance: Prior surrogate guidelines focused on qualitative criteria for a valid surrogate; this study presents a framework for optimal surrogate experimental design. Additionally, the results quantitatively demonstrate the importance of knowing surrogate-pathogen relationships for both resistance and variability when establishing a valid surrogate.

P3-155 The History and Current Use of Probabilistic Exposure Assessment in Dietary Assessments

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Introduction: This presentation provides a review of the 40-year history and the present use of Probabilistic Exposure Assessments for chemical hazards in foods.

Purpose: To provide a comprehensive review of the use of probabilistic exposure assessment in industry and in government and the variations in the practice that result from the different reasons why a chemical is present in food.

Methods: The presentation reviews the history of use and associated guidance for the conduct of PEAs in food. The characterization of the use of PEAs is framed by different categories based on the source and mechanism by which a chemical enters foods: natural and anthropogenic contaminants of crops; deliberately added fertilizer, pesticide, feed, veterinary and food additives; those formed during storage or processing; and migrants from food contact materials. Example calculations provide generic relationships between the source categories and the resulting nature of the PEA models.

Results: The applications (i.e. the nature of the calculations and data needs) of PEAs are fundamentally linked to the point at which the chemical first occurs and the reason for its presence in food. The presentation provides a roadmap to the creation of PEA model structures that are linked to the source

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and mechanism by which a chemical enters or first occurs in the supply chain for the ultimate food product. A key factor relates to the extent of blending of raw agricultural commodities and its impact on chemical exposure of consumers.

Significance: The presentation provides an up-to-date and comprehensive review of the practices of PEA for chemical exposures in foods. Understanding the current state of the art is critical to the food industry and the global regulatory community for meeting the expectations of professional practice in understanding the risks and the safety of foods.

P3-156 A Screening Risk Assessment Method to Prioritise Management of Imported Toxic Plants Restricted for Food Uses

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Introduction: The Australia New Zealand Food Standards Code (FSC) defines 186 different plant and fungi species that are prohibited for sale as food due to their production of natural toxins, that when consumed may be detrimental to human health. Concerns prohibited plants were being imported to New Zealand for food purposes or food-based traditional medicines necessitated a prioritisation tool to direct risk management resources to the plant or fungi species of highest risk from the FSC.

Purpose: To develop a screening assessment methodology to prioritise the highest risk plant and fungi species so to most efficiently direct risk management and compliance resources.

Methods: A risk ranking methodology was developed to define a priority list of plants and fungi. This method considered data that may be deficient for assessing hazard and exposure of plants and fungi. To characterise the hazard, a scoring matrix was defined to balance acute and chronic toxicity of natural toxins. Assessment of exposure was scored based on the risk the species would be consumed, whether the toxins were detoxified with normal processing and whether there were legitimate non-food uses to justify the import of the plants.

Results: Scores from the hazard and exposure characterisation were added to obtain the risk ranking. Each genus and/or species was scored out of 15, with a cut-off score of 11 then used to focus on species with notable acute or chronic toxicity, and for which evidence indicated would present a risk of being imported for food uses.

Significance: The scoring matrix and considerations used for this risk ranking may be applicable for prioritising other potentially hazardous food types.

P3-157 Prediction of Spore Germination and Radial Growth of Fungi in Dairy Products as a Function of Temperature, pH, Water Activity, Lactic and Propionic Acids

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Introduction: Filamentous fungi can spoil foods leading to significant waste and economic losses. Predictive microbiology is mainly focused on the assessment of spoilage and sanitary risks linked to bacterial activity, while there is a lack of information about the growth of fungi in foods.

Purpose: To study the diversity of filamentous fungi physiology, the impact of the main abiotic factors was assessed on the spore germination and radial growth of fungal species isolated from food or industrial environment.

Methods: The impact of temperature, water activity, % CO2, pH, lactic and propionic acids were assessed in PDA medium for sixteen fungal species. Almost 8 levels were tested for each factor (10 levels as median value) with at least 5 of them with an observed growth (8 levels as median value). A classical twostep modeling approach was used: i) kinetic modeling of the thallus growth or spore germination, and ii) modeling of kinetic parameters using Gamma (ɣ) concept. Validations of predictions were performed using challenge-tests.

Results: A wide diversity of physiological responses was observed through a large range of growth limits for each factor. Mycelial growth was observed at the lowest temperature (5°C) and no strain was able to grow at 45°C. The pH did not affect the growth of some species whereas some had an optimal and a minimal value. For the lactic or propionic acid and the % CO2, minimum inhibitory concentration (MIC) were estimated. Interestingly, optimal values were observed for some species/acid combinations. Growth predictions were validated for various species for temperature and acid concentrations on brioche and various dairy products.

Significance: Predictive mycology tools were improved with new models adapted to predict the response of molds to diverse environmental conditions. Graphical user interface allows one to take into account the diversity of fungal physiology to identify hazards or to assess food shelf life.

P3-158 Prediction and Interpretation of Bacterial Population Behavior in Food by Data Mining

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Introduction: Although bacterial population behavior has been investigated in various foods in the past 40 years, it is difficult to obtain desired information from the mere juxtaposition of experimental data.

Purpose: The objective of this study is to predict the changes in the number of bacteria and visualize the effects of pH, aw, and temperature using a data mining approach.

Methods: Population growth and inactivation data on eight pathogenic and food spoilage bacteria under 5,025 environmental conditions were obtained from the ComBase database (www.combase.cc), including 15 food categories, and temperatures ranging from 0°C to 25°C. "Time (h)," "Temperature (°C)," "pH," "a_w," "Initial cell number (log CFU/g)," "Food category," "Food name," and "Organism." are used to predict cells density over time. The eXtreme gradient boosting tree was used to predict population behavior.

Results: The root mean square error of the observed and predicted values was 1.23 log CFU/g. The data mining model extracted the growth inhibition for the investigated bacteria against aw, temperature, and pH using the SHapley Additive eXplanations value. Categorical data such as organism, food category, and food name also contributed to the construction of the model to some extent in the developed model.

Significance: The data mining approach allowed us to model and reveal the multidimensional relationship between bacterial population behavior and the food environment. We showed that a data-driven approach to analyzing accumulated data could be useful for addressing food safety issues.

P3-159 Expected Health Risk from Consumption of Pesticide Residues on Produce

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Introduction: Questions are periodically raised about the potential health risks associated with consumption of food with pesticide residues.

Purpose: This study aims to estimate pesticide exposures for consumers of select fruits and vegetables in the U.S. and to characterize the associated non-cancer human health risks.

Methods: Twelve produce types were identified through the 2022 Environmental Working Group "Dirty Dozen" list. The single most commonly detected pesticide was identified for each produce type, and the USDA Pesticide Data Program database was then used to determine representative residue concentrations for each pesticide-produce combination. Whole population-based mean dietary consumption information for each produce type was extracted from the U.S. EPA Exposure Factors Handbook and used to calculate dietary exposure to each pesticide-produce combination for adults and children. The pesticide-specific U.S. EPA chronic oral population-adjusted (PADs) or reference doses (RfDs) were then used as health benchmarks to evaluate non-cancer risks of consuming each produce type and to determine the daily amount of each produce type that is acceptable to consume.

Results: The estimated daily exposure was below the corresponding RfD for all exposure scenarios, with margin of safety (MoS) values lowest for nectarines and highest for cherries and celery. The MoS for adults across all produce types ranged from 130 to 1,800,000 using the median reported pesticide residue concentrations and from 130 to 720,000 at the 95th percentile residue concentrations. Based on the median reported residue concentrations, excessive produce-specific pesticide exposure is unexpected as the weight of produce that would need to be consumed on a chronic basis, even among children, far exceeds typical dietary intake.

Significance: Although exposures to pesticide residues from produce are typically low and below acceptable chronic exposure limits for non-cancer effects, a varied diet is always recommended.

P3-160 Challenge Tests to Study Inactivation Potential and Kinetic Parameters (ISO 20976-2:2022)

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Introduction: It is the responsibility of Food Business Operators to control microbiological hazards in foods and to manage microbial risks according to the general principles of the Codex Alimentarius on food hygiene. Challenge test is one of the recognized approaches used to validate control measures within the HACCP system, as well as to assess microbiological safety and quality of food, food production processes, food storage conditions and food preparation recommendations for consumers.

Purpose: In agreement with already available and valuable guidance documents that exist worldwide, the aim of the ISO20976 standard series is to provide general requirements and guidelines for conducting challenge tests on food and feed products with a distinction made between studies targeting growth (ISO20976-1:2019) or inactivation (ISO20976-2:2022) in a specific micro-organism/food combination

Methods: Within the frame of the ISO, members from all over the world collaborate to create internationally recognized documents providing requirements, specifications, guidelines or characteristics that can be used consistently to ensure that products, processes and services are fit for purpose. ISO/TC34/SC09/WG19 is an ISO working group operating in the field of microbiological analysis of the food chain. It comprises experts from the food industry, food technology institute, food testing laboratory, research center and regulatory bodies.

Results: The ISO 20976-2:2022 Standard for conducting challenge tests to study inactivation potential and kinetic parameters was published in November 2022 and provides recommendations and guidelines on several topics such as the number of batches to be tested, the selection of strains for the challenge test, the inoculation procedure and the rules for interpretation. WG19 is currently working on an additional standard for the determination and use of cardinal values in predictive microbiology (project ISO 23691).

Significance: General and consensus documents on best practise for conducting challenge tests will ensure harmonisation of practices to facilitate data interpretation and trade between stakeholders from different countries.

Poster

P3-161 Predictive Modeling of Wheat Flour Safety Recall Behaviors and Recall Awareness

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❖ ***Developing Scientist Entrant***

Introduction: Numerous recalls and outbreaks of *Salmonella* and Shiga toxin-producing *Escherichia coli* were associated with raw wheat flour products. A previous survey showed that many consumers were unaware of these recalls or outbreaks.

Purpose: The aim of this study was to identify the top predictors of consumer awareness of recalls and their recall practices.

Methods: We analyzed previous consumer survey data (n=918) illuminating risk perception of flour products, behaviors during a hypothetical flour recall, and demographic characteristics of consumers. The survey data were overlaid with the number of flour-related outbreaks from 2008 to 2018 in the United States. Two predictive model-ensembles were built to predict consumers' awareness of flour recalls and their recall practices. The model-ensembles contained (1) a random forest, (2) a neural network, and (3) a logistical regression (for binary data). Model performance and the marginal effects of features on the predicted outcomes were assessed. Relative feature importance from each model-ensemble was used to select top predictors.

Results: Surprisingly, the number of flour-related outbreaks in a participant's geographical state does not influence a participant's awareness of flour recall or recall practices. Instead, younger consumers, and consumers with a lower likelihood for perception of a recall of flour are top predictors that indicate a trend toward a higher probability of being aware of recalls. Consumers who view raw chicken as a microbiological food safety risk and rarely engage in eating or playing with raw cookie dough are top predictors for a higher probability of having good flour recall practices. Assessment of marginal effects for predicting good flour recall practices indicates an interaction between paying attention to flour warning messages and cleaning surfaces after handling flour.

Significance: The findings will support the development of future education and communication materials that are intended to increase consumer awareness of flour food safety and flour recall best practices.

P3-162 Random Forest Models of Food Safety Behavior Frequencies during the COVID-19 Pandemic

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Introduction: The COVID-19 pandemic affected the frequency of consumer food safety behaviors, such as washing produce and washing hands. Furthermore, little is known about the top predictors of consumer food safety practices during the pandemic.
Purpose: The aim of this study was to (1) identify the top predictors of consumer food safety behavior frequencies before and during the COVID-19 pandemic and (2) investigate how top predictors may have changed over time.
Methods: We analyzed consumer survey data across nine months (average n=728) that featured: (1) risk perceptions and knowledge sources of COVID-19 and food safety, (2) trusted sources of information, and (3) self-reported food safety behavior frequencies before and during the COVID-19 pandemic. Random forest models were constructed to predict two food safety behaviors and 12 food safety behavior frequencies. Relative importance was used to select top predictors in subsequent models, and partial dependency plots were constructed of top predictors to interpret the models. Model performance and importance ranking correlations between months were assessed.
Results: Food safety risk perceptions and COVID-19 risk perceptions were the top predictors of food safety practices, with the number of people in the household being the next top predictor. Higher risk perceptions of getting foodborne illness or COVID-19 were associated with higher frequency of food safety practices. The ranking of top predictors varied over time ($\tau < |0.5|$). When COVID-19 cases were spiking in the United States, COVID-19 risk perceptions ranked higher among top predictors of food safety practices than food safety risk perceptions ($r_{tet} = 0.9$). Models including only the top predictors had an equal performance with models including all features (mean error <|0.07|, AUC >=0.70).
Significance: Our findings suggest that COVID-19 and food safety risk perceptions are top predictors of consumer food safety behaviors. Future consumer food safety educational interventions targeting at risk perception changes can potentially improve behavior change.

P3-163 Estimating the Healthcare Cost of Foodborne Disease (FBD) from Electrical Medical Records (EMRs)

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Introduction: Electronic Medical Records (EMRs) allow for the derivation of more comprehensive and direct estimates for healthcare costs associated with foodborne illness.
Purpose: This research aims to estimate the healthcare costs associated with FBD, differentiated by hospital settings (e.g., outpatient, inpatient, emergency, other).
Methods: We acquired all patients' EMRs from a large Midwestern medical system between 2011 and 2020. Using this data, we aggregate encounters related to likely foodborne illnesses into episode/illness-level observations. This yields a final sample of 1,664,309 episodes. Approximately 2.6% of episodes are associated with one or more FBDs. We then we estimate mean episode costs and use fixed effect regression to analyze the effect of hospital visit setting on the healthcare cost of FBD. A Hausman specification test is performed to confirm the consistency of the fixed effect model.
Results: Preliminary estimates for the average cost of an episode of FBD were $19797, with a standard error of $285. However, the median cost was $2799 (25% percentile is $445, 75% percentile is $13492). The right-skewed shape suggests a need for taking a log of the healthcare cost. These costs vary by the initial visit to the hospital setting. Most hospital settings (defined by the settings of the first visit) are outpatient (70.9%), while emergency and inpatient have similar proportions. (6.8% vs. 6.7%). The fixed-effect panel regression indicated that one diagnosed FBD related to a 101% increase in healthcare cost compared to the episodes without diagnosed FBD (p<0.01). In addition, for the episodes with emergency settings on the first visit, the increment in FBD costs is 16% and 32% smaller than the increments for the outpatient and inpatient settings respectively (p<0.01). The Hausman test supported the use of the fixed effect model for consistency.
Significance: This research advances efforts to estimate healthcare costs of FBD more accurately.

P3-164 Machine-Learning Approach to Classify Raw Milk Based on Mesophilic and Thermophilic Spore Concentration Using Farm Survey and Weather Data

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Developing Scientist Entrant

Introduction: Mesophilic and thermophilic sporeformers in raw milk are associated with quality issues in shelf-stable dairy products and ingredients, particularly dairy powder.
Purpose: The goal of this project is to apply a machine learning approach to classify raw milk based on the mesophilic (MSC) and thermophilic (TSC) spore concentration.
Methods: A dataset for 144 raw milk samples was collected from 12 dairy farms in NY and was composed of (i) farm management survey data, (ii) microbiological data, and (iii) weather data. Four classification models were developed, each with four different outcome variables based on spore levels for dairy powder (60, 100 CFU/g for MSC and 10, 40 CFU/g for TSC), calculated assuming the yield of 12.8g whole milk powder per 100mL milk. Training and testing sets were split at 7:3 with stratification to account for class imbalance. Each classification model was trained and tuned using both random forest and xgboost classifiers. The best model for each classification outcome was selected based on accuracy and was further evaluated on an independent testing set.
Results: Random forest slightly outperformed xgboost in all trained models. For each classification outcome (60, 100 CFU/g for MSC and 10, 40 CFU/g for TSC respectively), the best trained model had an accuracy of 81.1%, 69.2%, 78.0%, 75.0%, respectively. Validation results using these best models showed that highest accuracy was achieved at 81.8% for classifying raw milk to meet MSC specification of 60 CFU/g whole milk powder. Random forest models consistently ranked forestripping and pen cleaning frequency as top farm-level predictors while xgboost models consistently ranked precipitation, air temperature and somatic cell counts as top predictors.
Significance: The model developed can help guide farm management practices as well as selection of raw milk for powder manufacturing to reduce quality issues due to mesophilic and thermophilic sporeformers.

Poster

P3-165 Evaluating the Effectiveness of Sampling Plans and Locations in Multi-Harvest Commodities through the Development of a Farm-to-Packinghouse Simulation for Tomatoes

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Developing Scientist Entrant

Introduction: Current commercial practices may suggest test and reject (sampling) plans for tomatoes. If implemented, the effect that these plans have on total adulterant cells reaching the system endpoint (endpoint TACs) is unknown. Furthermore, little guidance is available on where and how to sample along the farm-to-packinghouse process.

Purpose: To identify best sampling plan and process stage combinations that reduce the endpoint TACs for different contamination scenarios.

Methods: A 42-day season with a mass of 132,000 lb tomatoes and a contamination concentration of 1 CFU/lb was modeled. Four generic contamination spreads (Widespread-100%, 10%, 1%, and 0.1% clusters), six sampling plans (60-tomatoes: 15,600g, 20-tomatoes: 5,200g, 6-tomatoes:1,560g, 2-tomatoes: 520g, 60-tomato-mash: 1,500g, and 20-tomato-mash: 500g), and four processing stage (preharvest, harvest, receiving, and packing) combinations were modeled for 96 total scenarios. The scenarios were assessed based on their ability to detect contamination and reduce endpoint TACs compared to no sampling (relative efficacy).

Results: As the initial degree of clustering increases, the efficacy of sampling plans decreases. 98.1% maximum efficacy for widespread-100% contamination vs 12.3% maximum efficacy for 0.1% cluster contamination. For the widespread, 10%, and 1% clusters the best sampling locations are at harvest and receiving, with efficacy ranging between 39.9% to 98.1% for the most effective sampling plans, whereas for the 0.1% cluster, the best sampling location shows to be packed product sampling, 12.3% relative efficacy. The model suggests that for the widespread and 10% cluster contaminations, sampling plans with larger masses such as 60 and 20 tomatoes had the greatest efficacy. For the 1% and 0.1% clusters sampling plans that had the greatest number of grabs, 60 tomatoes and 60-tomato mash.

Significance: This work can help stakeholders decide how and where in the farm-to-packinghouse process to sample based on the needed performance, hypothesized clustering of contamination, and amount of product is is feasible to test.

P3-166 Predictive Modelling of Lactic Acid Bacteria and *Listeria monocytogenes* in Canastra Cheeses Stored in Active Packaging with Silver Nanoparticles

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Introduction: Canastra cheese is an artisanal product of cultural and economic importance produced in the Serra da Canastra region, Brazil with unique flavor characteristics conferred by indigenous lactic acid bacteria (LAB). Because it is produced from raw milk, it may be associated with pathogens such as *Listeria monocytogenes*. Active packaging based on silver nanoparticles has been suggested as promising alternatives to raw milk cheeses due to their antimicrobial activity.

Purpose: To evaluate the behavior of *L. monocytogenes* and LAB during the storage period of artisanal Canastra cheeses in an active packaging system with silver nanoparticles.

Methods: Canastra cheese samples (60 g) were superficially inoculated with a *L. monocytogenes* cocktail (ATCC 7644, CLIST 3970, CLIST 4161; final counts at 3 log CFU.g^{-1}). *L. monocytogenes* and LAB were quantified during storage of 38 days, at 5, 10, and 20°C in cheeses packaged or not with the active silver nanoparticles system (3,000 ppm active based on silicon dioxide and silver). The Weibull predictive model was adjusted to the data to obtain the mathematical parameters of inactivation.

Results: Based *L. monocytogenes* and LAB counts over the storage, the time required for the reduction of the population to occur in a logarithmic cycle was calculated. No regularity of behavior between the traditional and active packaging for *L. monocytogenes* or LAB was observed. At 5°C, the active packaging showed greater *L. monocytogenes* inactivation compared to the traditional packaging (delta parameter values of 53.9 and 168.1 min^{-1}, respectively), while at 10°C, the opposite was observed (delta parameter values of 144.1 and 44.3 min^{-1}, respectively). At 20°C, similar values of the delta parameter (30.5 and 38.4 min^{-1}) were observed.

Significance: The active packaging tested allowed the survival of indigenous LAB in Canastra cheese and its effects against *L. monocytogenes* varied with the temperature.

P3-167 *Salmonella enterica* Growth and Survival Kinetics in Fresh-Cut Purple Cabbage Stored at Different Relative Humidity and Temperatures

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Introduction: Salmonellosis linked to contaminated fresh-cut vegetables remains an important concern globally. *S. enterica* survival is impacted by Relative humidity (RH), but the effects of RH on the survival kinetics of this pathogen in fresh-cut-purple cabbage remains unknown.

Purpose: This research investigates the growth and survival kinetics of *S. enterica* in fresh-cut-purple cabbage stored at 7, 14, and 21°C at 15, 35, 65 and 95% RH.

Methods: Samples were inoculated with a cocktail of *S. enterica* (*S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Agona, *S.* Anatum) at ~3.5 log CFU/g and held in desiccators over saturated salt solutions. *Salmonella* cells were enumerated onto Xylose Lysine Deoxycholate Agar over 144 h storage. Survival curves were fitted using the Solver add-in for Microsoft Office Excel v1.6.

Results: S. enterica increased (1 log) after 144 h at 21°C at 95% RH, did not change at 65% RH and decreased (1.5 log) at 35% and 15% RH. Counts did not change after 144 h at 14°C at 95% RH but decreased (1.3 log) at 65% RH. Counts decreased (1.8 log) in samples stored at 7°C at 95% and 65% RH after 144 h and 120 h, respectively. At 14 and 7°C at 35% RH counts decreased (1.8 and 1 log, respectively), after 96 h. The log-linear model showed a good fit for *Salmonella* inactivation at 15% and 35% RH for all temperatures (R^2>0.93), and at 65% RH at 14 and 7°C (R^2>0.91). A log-linear with shoulder model showed good fit at 7°C at 95% RH (R^2 0.98), while at 21°C, the best fit was by the Baranyi and Roberts model (R^2 0.95). Data from 21°C at 65% RH and 14°C at 95% RH did not fit any model.

Significance: These findings will assist in developing risk management strategies for *S. enterica* in fresh-cut cabbage.

Poster

P3-168 Unraveling the Microbial Communities in the Ginger Bug (starter) from Organic *Zingiber officinale* Roscoe Using Culture Dependent and Independent Methods

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Introduction: The ginger beer starter is a fermented culture of native microorganisms of ginger (*Zingiber officinale* Roscoe) used to produce ginger beverages. The starter defines the quality and safety of the final product. Little is known about the microbial diversity in the starter production.

Purpose: To characterize the microbiota in ginger bug (*starter*) from organic ginger through culture dependent and independent methods.

Methods: The ginger microbiota was activated to bug using grated organic ginger previously sanitized (8%; w/v), organic crystal sugar (8%; w/v) and sterile mineral water (500mL). The mixture was maintained under aerobic conditions (25°C) for 96h with addition of ginger and sugar (4%; w/v) every 24h. Lactic acid bacteria and acetic acid bacteria were evaluated by plating in differential medium. DNA was extracted using the Power Food DNA isolation kit. The Illumina TruseqDNA Sample Preparation v2 kit was used for the 16S rRNA sequencing and QIIME2 v.2021.2 to assign the taxonomy in the high-quality-readings. Three batchs were perfomed. All analyses were done in triplicate. XLSTAT v.2020.1.3 software was used for statistics.

Results: Lactic bacteria increased around 1 log CFU/mL after 96h, reaching counts of 4.33 log, while acetic bacteria decreased by about 2 log CFU/mL, reaching counts of 7.74 log CFU/mL after 96h. Four phyla were identified in the bug (after 96h), being *Proteobacteria* and *Firmicutes* dominant, followed by *Acidobacteria* and *Actinobacteria*. *Lactobacillaceae*, *Acetobacteraceae, Pseudomonadaceae, Ruminococcaceae, Koribacteraceae, Enterobacteriaceae*, were the main bacterial families initially present followed by, *Thermoactinomycetaceae, Methanobacteriaceae* after 48 and 96h. *Lentilactobacillus, Pseudomonas, Enterobacter, Lelliottia, Acetobacter, Candidatus Koribacter, Caproiciproducens,* were identified before the fermentation. After 48 and 96h, *Pseudocitrobacter* and *Methanobacterium* were identified. None of the identified microorganisms pose a risk to human health.

Significance: Results characterize the microbiota of organic ginger bug. Findings suggest that organic ginger bug has a microbiota safe to be used as *starter* for ginger beverages.

P3-169 Survival of *Salmonella enterica* in Chocolate Made with Contaminated Coconut Flakes during Storage at Different Temperatures and Relative Humidities

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Introduction: Chocolate has been involved in recalls and outbreaks due to contamination with *Salmonella enterica*. Contamination of chocolate occurs primarily when ingredients containing *S. enterica* are incorporated in chocolate and the cells survive processing and storage. Few studies have assessed *Salmonella* behavior in chocolate products during storage.

Purpose: This study investigated the survival of *Salmonella* in chocolate made with contaminated coconut flakes stored at different temperature and relative humidity (RH) conditions.

Methods: Tempered chocolate paste was mixed with dehydrated coconut flakes contaminated with a *S. enterica* cocktail (*S.* Typhimurium, *S.* Enteritidis, *S*. Newport, *S.* Oranienburg and *S*. Montevideo; ~7 log CFU/g). The chocolate mixture was molded into 3 cm × 3 cm × 2.3 cm portions and packaged in biaxially oriented polypropylene with a known water vapor transmission rate. This packaging approximated the most common commercial packaging material used for chocolate. Packaged chocolate samples were held in desiccators over nitrate chloride and potassium chloride saturated salt solutions (65 and 100% RH, respectively) at 7 and 25°C. *Salmonella* were enumerated over 8 days of storage onto *Salmonella-shigella* (SS) agar after every 24 h (detection limit 1.5 log CFU/g). Statistically significant differences were considered to occur if $P < 0.05$.

Results: Salmonella counts did not change significantly over 4 days of storage, regardless of the temperature or RH. After 8 days of storage at 7°C, *Salmonella* counts decreased by 1.2 ± 0.2 log CFU/g at 100% RH and by 1.5 ± 0.3 log CFU/g at 65% RH. At 25 °C, *Salmonella* counts decreased by 2.2 ± 0.3 log CFU/g at 65% RH and by 1.2 ± 0.1 log CFU/g at 100% RH after 8 days of storage.

Significance: These findings are important in making risk management decisions for chocolate products found to be contaminated with *Salmonella*.

P3-170 Microbial Groups Revealed By High-Throughput DNA Sequencing in Fresh Edible Red Mini-Roses (*Rosa chinensis* Jacq.) from Different Farming Systems

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Introduction: Flowers became raw ingredients in food dishes around the world. Mini-roses (*Rosa chinensis* Jacq.) are among the preferred flowers for fresh consumption due to their light texture and citrus taste. The farming system may affect the microbiota in flowers. Little is known about the indigenous microorganisms in mini-roses, regardless of the farming system.

Purpose: To assess the microbiota in fresh red mini-roses farmed in biocompost and manure systems using metabarcoding (16S rRNA and 18S rRNA) sequencing

Methods: Mini-roses were farmed in biocompost derived from discarded fruits or in animal manure systems for 28 days (commercial maturity stage). A PowerSoil Pro (Qiagen) DNA isolation kit was used for DNA extraction. 16S rRNA (V3-V4) and 18S rRNA libraries were sequenced using a Nextera XTindex kit (Illumina, USA) on an Illumina NovaSeq6000 PE 250 platform (IlluminaInc., USA). High-quality readings were analyzed using Qiime2 (v2022.2.0). Reads were mapped against the reference 16S rRNA database (SILVA 138) and reference ITS (UNITE) for taxonomic assignment (ByMyCell -Genomics made simple, Brazil). Statistical analyzes were performed using XLSTAT 2020.1.3 ($p<0.05$).

Results: Dickeya and *Massilia* were the prevalent bacterial genera in mini-roses farmed in biocompost and animal manure, with no significant difference ($p>0.05$). On the other hand, *Pseudomonas* had higher abundance in mini-roses farmed in biocompost (13.22 vs 5.59%), while *Acinetobacter* had higher abundance in mini-roses farmed in animal manure (11.82 vs 3.86%). Regarding 18S rRNA gene amplicon, the analysis showed *Bipolaris* sp as dominant in mini-roses farmed in both systems, but with higher abundance in animal manure (85.74 vs 95.61 %) ($p<0.05$)mini-roses farmed*Eurotiales* (1.82 vs 0.13%) and *Cladosporium exasperatum* (3.53 vs 0.40%).

Significance: Results characterize the indigenous microbiota in a widely raw consumed edible flower. Foodborne pathogens were not identified in mini-roses, regardless of the farming system.

P3-171 Metagenomic Analysis of Microbial Biodiversity and Its Associated Resistome Profile within the Melon Agroecosystem

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial resistance of bacteria poses a significant threat to public health, and understanding the presence and spread of antibiotic resistance genes (ARGs) in agroecosystems is crucial for addressing this issue. Next generation sequencing (NGS) technologies can provide detailed information about the microbiome and resistome in these environments.

Purpose: Through NGS technologies analyze the associated microbiome and resistome in a melon agroecosystem.

Methods: DNA samples (n=32) were processed from soil and workers' hands and sequenced for taxonomy and resistome determination. Oxford Nanopore Technologies was used to analyze metagenomic samples from soil and workers' hands of melon farms. The SQK-RBK004 kit was used for a 14-hour run, followed by bioinformatics analysis using EPI2ME, WIMP, and ARMA. Sequences were processed using Nanochop, Nanoplot, Kraken2, Mothur, and EPI2ME labs to determine taxonomy and ARGs. Results were visualized using MEGAN, Graphpad Prism 9 and Krona.

Results: Metagenomic DNA samples yield 631,625 demultiplexed reads. Taxonomy and ARGs analyses were performed with an alpha of 0.5 and a minimum accuracy of 80%. Quality score was 10.85 and an N50 of 1,214 bases. Results showed that soil samples had high abundances of *Pseudomonas fulva* and *Escherichia coli*, with resistance genes *mgrB, PmrF*, and *kdp*. Samples from workers' hands showed greater microbial diversity, with high abundances of *Pseudomonas aeruginosa*, *Salmonella enterica* and *E. coli*, all carrying various resistance genes. The study also exhibited a diverse population of Archaea.

Significance: NGS in agroecosystem research can provide high-resolution and information about the microbiome and resistome in these environments. This study can aid in rapid and accurate monitoring of agroecosystems with high food production, and inform food safety and security efforts by identifying potential pathogens and ARGs present in fresh food.

P3-172 Towards a Biocontrol Solution forSTEC in Romaine Lettuce: Microbial Diversity Among Soil Samples Reveals a Disparate Taxonomic Structure from Eastern and Western U.S. Leafy Green Fields

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✦ ***Undergraduate Student Award Entrant***

Introduction: Since 2017, leafy greens have been heavily implicated in a multitude of STEC outbreaks. Solutions for the control of STEC persistence and spread are essential to mitigate future outbreak risks.

Purpose: To understand differences in soil biodiversity between eastern and western U.S. leafy green growing regions and to identify a potential STEC biocontrol.

Methods: DNA was isolated from soil samples near leafy green growing operations collected either from Yuma, Arizona (9) (western) or Maryland (4) (eastern). Libraries were constructed using an Illumina Flex kit and shotgun metagenomic sequencing was done on an Illumina NextSeq 2000 platform. Community-level physiological profiling (CLPP) was obtained using Biolog EcoPlate™. CLLP data was analyzed for average well color development (AWCD) and Shannon diversity index (H). Lastly, soil microorganisms were isolated and identified for potential biological control use with Biolog GenIII microbial identification system.

Results: Substantially greater genetic biodiversity was observed in western soil samples relative to eastern soil samples. Notably, several gram-positive and gram-negative genera including *Nitrosomonas, Sinorhizobium, Nitrobacter, Lysobacter, Novibacillus,* and *Aeromicrobium* were identified only from western soil samples. The overall metabolic activity (AWCD) and physiological diversity (H) of soil communities at 72h was slightly higher in western soil samples, but not significantly different from those in eastern soil samples (P=0.2409 and P=0.671, respectively). Increased metabolic activity was observed in β-methyl-D-Glucoside and phenylethylamine as carbon sources in eastern soil communities, and DL-α-Glycerol Phosphate in western soil communities. Thirty isolates from multiple *Bacillus* spp., *Pseudomonas fluorescens* and *Rothia amorae* have been identified, some of which belong to species well established in the biocontrol of enteric pathogens.

Significance: Clear taxonomic differences can be seen between soil microbiomes from these disparate but agriculturally important locales. The preliminary data here point to the potential to identify a biocontrol isolate effective against STEC from western region soils.

P3-173 Microencapsulation Protects the Survival of Probiotic Bacteria during Heat Treatment

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Introduction: Microencapsulation is considered as an effective technique to prolong probiotic survival during exposure to harsh conditions (i.e., heat treatment).

Purpose: The aim of this study was to examine the survival of encapsulated cells after heat treatment.

Methods: *Lacticaseibacillus casei* Shirota and *Lacticaseibacillus rhamnosus* GG were encapsulated (8 log CFU/mL) in a whey protein isolate-gum arabic coacervate matrix (WPI:GA). Consequently, the encapsulated cells were added in a food-model (TSB without glucose) and heat treatment was applied of 50, 55, 60, 65°C for 0, 1, 5, 10, 15 and 20 min. Free cells were used as controls. The experimental data (counts in log CFU/mL) were fitted using the modified Weibull model to describe the survival kinetics of bacterial population after treatment.

Results: Results showed that heat treatment at 50°C for each holding time, did not affect the encapsulated cells that were remained at 8 log CFU/mL, whereas treatments in higher temperatures resulted in cells decline that reached 5 log CFU/mL. Free cells decreased to under detection limit (<1 log CFU/mL), after treatment at 55, 60, 65°C for 20 min. The modified Weibull model provided a good fit to the survival data of the bacteria (R^2>0.960). As regards *δ* rate, lower rate indicates higher death rate of bacteria, consequently, the lowest *δ*-values were obtained for the samples heated at 60 and 65°C for 20 min. Also, since δ parameter corresponds to the time of the first decimal reduction of the population, the encapsulated cells provided the highest survival rates, especially at 50°C, where no decimal reduction was observed during heat treatment.

Significance: It can be concluded that the WPI:GA coacervate can be efficient in protecting the sensitive probiotic cells during heating at pasteurization temperatures.

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Poster

P3-174 Microbial Diversity of Chill-Stored Mussels (*Mytilus galloprovincialis*) Using 16S Next Generation Sequencing

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Introduction: Mussels are among the most well-known seafood worldwide. However, little is known about the microbiota succession of mussels with and without shell during commercialized storage conditions.

Purpose: The present work aimed to study the microbiota succession, as well as to determine the dominant bacteria of mussels (*Mytilus galloprovincialis*) with and without shell during storage at commercialized temperature conditions.

Methods: Mussels with and without shell were stored at commercialized conditions (2°C and 4°C for mussels without shell and 4°C for mussels with shell). The end of shelf-life, examined by sensory analysis, was determined at 6 days in all cases. A total of 25g for each product were collected from the initial, middle and the end of shelf-life for DNA extraction, followed by the amplification of V3-V4 region of 16S rRNA gene for metabarcoding analysis. Bioinformatic analysis was applied using the MR DNA ribosomal and functional gene analysis pipeline and the results were expressed as relative abundances (%).

Results: At the beginning of storage, a high bacterial biodiversity was observed in all cases, with *Leuconostoc*, *Acinetobacter*, *Corynebacterium* and *Psychrobacter* being the most representative bacteria in fresh mussels without shell. On the other hand, *Candidatus fritschae*, *Marinobacterium* and *Corynebacterium* were the most abundant bacteria in fresh samples with shell. At the end of shelf-life (D 6), the genera *Psychrobacter* and *Pseudoalteromonas* were by far the most abundant genera in mussels without shell in both storage temperatures (2 °C and 4 °C), while *Vibrio* and *Pseudoalteromonas*, followed by *Phychrilyobacter* dominated in products with shell, even though the presence of *Marinobacterium* and *Francisella* was also noteworthy.

Significance: The handling and storage of mussels affected the microbiota profile during storage at commercialized conditions. This study was funded by an HORIZON Project DiTECT (861915)

P3-175 Bacterial Communities of European Seabass (*Dicentrarchus labrax*) at Chilled Temperatures Using 16S Metabarcoding Analysis

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Introduction: The microbial communities' succession and domination of Specific Spoilage Organisms (SSOs) in seafood during storage depend on various factors such as the type of the product, the storage conditions, and the microbial interactions.

Purpose: This study examined the effect of primary processing on the microbiotas' composition as well as the type of spoilage bacteria that dominate, during chilled storage of whole, gutted and filleted seabass

Methods: Whole and gutted seabass were stored at 0 °C for 18 days and filleted seabass at 2 °C for 12 days. The end of shelf-life was determined by sensory analysis, and it was 15 days for whole and gutted and 10 days for filleted seabass. Samples of 25g flesh for each product were obtained from the initial, middle and final (end of shelf-life) storage stages for DNA extraction. Then, 16S metabarcoding analysis of the DNA samples was carried out in order to obtain the relative abundances (%) of the microbial consortium present in the three products.

Results: Initially, *Thermus* was present in the top three genera along with *Kluyvera* and *Lelliottia* for whole, *Propionibacterium* and *Rahnella* for gutted and *Burkholderia* and *Luteibacter* for the filleted seabass. In the middle storage stage (day 9 for whole and gutted, day 6 for fillet) *Pseudomonas* prevailed with relative abundances of 69,2% (whole), 73,86% (gutted) and 79,9% (fillet). *Shewanella* and *Serratia* follow *Pseudomonas* both in whole and gutted, whereas in fillet *Rahnella* (11,89%) follows first, while the presence of *Shewanella* (4,81%) was also noteworthy. At the end of shelf-life *Pseudomonas* was still the dominant spoilage genus, with *Shewanella* being also present in the top three of all the products.

Significance: Primary processing and storage affected the microbiota composition during chilled storage, but not the dominant spoilage microorganism which was *Pseudomonas* for all the products. This study has been funded by the DiYECT an HORIZON project.

P3-176 Validation and Implementation of Expanded Contextual Data through FDA's Genometrakr Network

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Introduction: FDA's GenomeTrakr (GT) program and laboratory network collects genomic data on foodborne pathogens from food, water, and environmental sources, contributing a key piece of the US's One Health approach for enteric disease surveillance. In 2022, FDA, CDC, USDA, and NCBI released a new data standard, the One Health Enteric package, that greatly expanded and standardized crucial pieces of contextual data required for interpreting genomic results for public health and regulatory application.

Purpose: Evaluate implementation, validation, and early data submitted using the new One Health Enteric package.

Methods: A web-based tool for validating the One Health Enteric package, was developed to improve the quality of metadata terms submitted to NCBI. Using a variety of NCBI queries (AWS Athena, Entrez API, etc.) we captured sample and sequence contextual data attributes for all GT submissions from 2013-present. We used Tableau Desktop and other graphical tools to visualize the resulting tables.

Results: The public GT Metadata Validation System now includes validation for the One Health Enteric Package, providing a quick and easy metadata check for laboratories prior to NCBI submission. A new suite of data visualization dashboards were created to summarize the adoption of expanded metadata attributes across the GT network. Improved standardization of sample description attributes enabled more precise, machine readable metadata, specifically when characterizing samples taken from facility inspections, food products and processing steps, and environmental samples like agricultural water surveillance. Analysis of genomic data coupled with improved contextual data will significantly strengthen data analyses used for source attribution and risk prediction models.

Significance: Early implementation of the One Health Enteric package shows significant improvement in public metadata attached to the whole genome sequence data of enteric pathogens collected by the GT network.

P3-177 The Next-Generation Tools for Risk Assessment and Precision Food Safety: Use of Shotgun Metagenomics Sequencing for Characterisation of Food and Investigation of Metagenome-Assembled Genomes

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Introduction: Next Generation Sequencing (NGS) technologies and the application of bioinformatic approaches are redesigning microbiology and their applications, covering aspects of food quality and precision food safety. Use of NGS protocols includes, among others, a deep understanding of the genomes of microorganisms in pure culture using whole genome sequencing (WGS) and importantly, metagenomics has allowed the extensive comprehension of the microbiota and microbiome of food. Microbial communities along food chains have increasingly been studied for describing the genetic diversity, functionality, and succession of spoilage microflora, foodborne pathogens and for studying functional microorganisms used for producing food by fermentation.

Purpose: In this study, water kefir (WK) was used as a model for evaluating the performances of sequencing approaches and the detailed description and resolution of complex microbial communities.

Methods: WK grains were used as a starter culture for the first fermentation conducted at 25°C for 48 hours followed by second fermentation at 25°C for 72 hours in sterile bottles containing organic ginger (~1g). Culture-dependent methods were used for the isolation of microorganisms and their DNA was extracted for WGS using Oxford Nanopore Technology platforms. Samples were described through full-length 16S rRNA gene sequencing and shotgun metagenomics coupled with software-controlled adaptive enrichment for improving the detection of rarer species identified during the fermentation with culture-dependent approaches.

Results: Several isolates were retrieved through culturing and were also identified as high-quality metagenome-assembled genomes (MAGs), including prominent probiotic species of the genus *Gluconobacter*, *Liquorilactobacillus*, *Lactiplantibacillus*, *Lentilactobacillus* and *Lacticaseibacillus*. Novel-identified species were sequenced from pure culture, providing a detailed characterisation of their genomes.

Significance: These next generation of tools are going to change radically the risk assessment approaches and methods for better pinpointing the quality of foods and also identifying contaminations and describing potential unknown threats with high resolution of pathogens present in food at the strain level using metagenome-assembled genomes.

P3-178 Effects of Manure-Based Biological Soil Amendments on Fresh Produce Phyllosphere Microbiome

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Introduction: Manure-Based Biological Soil Amendments (MBBSA) are applied to soils to improve plant growth and maintain soil quality and health. However, due to presence of naturally occurring food borne pathogens (FBP) originating from livestock intestinal reservoirs, the improper handling and application of MBBSA, especially in their raw or undertreated form, may result in contamination of fresh produce with foodborne pathogens.

Purpose: The objective of this study was to profile the phyllosphere microbiome of collard greens in response to MBBSA.

Methods: Experimental plots with (3 × 3 m) were amended with commercially composted chicken or dairy manure at control 0 kg/ha, or recommended 336 kg/ha right before planting. Collard greens were grown in the plots in triplicate. At the time of harvest, six leaf samples per plot were collected. Genomic DNA was then extracted from leaf washes, and V4 region of bacterial 16S rRNA gene was sequenced and analyzed.

Results: PCoA ordination plot of Weighted UniFrac distance showed distinct separation of cow manure-treated soils from untreated controls and chicken treated soils. Proteobacteria populations including gammaproteobacteria, and actinobacteria significantly enriched in produce treated with either MBBSA, while Verrucomicrobia abundance decreased ($p \leq 0.05$). Bacteriodetes was depleted in chicken manure treated produce.

Significance: Properly composted MBBSA may enrich phyllosphere bacterial phyla or families that frequent FBP linked to fresh produce belong to, however FBP members originating from manures are not detected.

P3-179 Standardized Workflow to Define the Biogeography of Genomic Diversity of Foodborne Pathogens

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Introduction: Foodborne pathogen response to selective pressures along the agricultural continuum to consumers has given rise to immense genetic and genomic diversity.

Purpose: Develop standardized approach to characterize and compare pangenomes of pathogens associated with different food commodities (e.g., *Listeria monocytogenes, Cronobacter sakazakii, Vibrio vulnificus*) to identify genes underlying environmental tropisms, which hold potential for informing new strategies to manage transmission.

Methods: Genome assemblies for bacterial isolates were obtained, along with sample metadata (e.g., source type: environmental, food-, or clinical-associated), from NCBI. Assemblies were processed for: (1) quality control (CheckM, GTDB-Tk), (2) annotation (Prokka), (3) pangenome investigation (Panaroo), and (4) feature identification (e.g., eggNOG-mapper2). A machine learning approach was employed to identify accessory gene enrichments by source types. Pangenome summary statistics and isolate source-enriched functions were compared across taxa.

Results: Pangenome expansiveness positively associated with genome size; i.e., *L. monocytogenes* < *C. sakazakii* < *V. vulnificus*. For each taxon, the total number of predicted genes per genome was, on average, greatest in isolates derived from samples of clinical-, then food-associated, and environmental origins (ANOVA $p<0.05$). Approximately 17.9% (1,485/8,281), 5.3% (699/13,269), and 1.1% (190/17,321) of genes within the pangenomes of the above respective pathogens were enriched by source type ($q<0.05$). The sets of enriched genes included those encoding metabolic features linked to persistence (i.e., heavy metal resistance in *L. monocytogenes* and *C. sakazakii*) and virulence (e.g., sialic acid metabolism in *V. vulnificus*), among other functions. Future research will aim to gain mechanistic understanding of these putative adaptations.

Significance: The reproducible bioinformatics workflow allows for rapid identification of genes and pathways with practical applications to promote food safety, such as providing new markers for molecular surveillance and potential targets for control, and can be extended to emerging pathogens of concern.

P3-180 Comparison of *Salmonella* Serotyping Analysis Tools on Metagenomic Sequencing Data of Low-Moisture Foods

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Introduction: The ability for *Salmonella* to survive in food commodities of low-water activity such as flour, and pistachios have been previously studied using a cocktail approach. However, determination of serovar survival has been difficult

Purpose: Here, we identify the survivability of *Salmonella enterica* serovars in pistachios and flour by metagenomic sequencing. Different analysis tools for precise detection of the multiple serovars were evaluated.

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Methods: Pistachios were inoculated with a 5-strain cocktail (serovars Anatum, Montevideo, Newport, Oranienberg, Senftenberg) and flour with a 4-strain cocktail (serovars Agona, Enteritidis, Mbandaka, Newport) and stored at 25°C at relative humidity (30% and 60%) for 365 and 168 days, respectively. Shotgun metagenomic sequencing was performed using Illumina Miseq. Raw fastq files were uploaded into Galaxytrakr and analyzed using the Short-Read Sequence Typer tool (SRST2) Seqsero2, Kraken2, and customized kmer and bettercallsal tools.

Results: Kraken2 was able to identify *Salmonella enterica* at species level in all samples. Kmer tool was able to detect Agona, Mbandaka, and Newport, but not Enteritidis in flour samples. SRST2 tool analysis revealed all serovars in flour and pistachio but only when all the reads from the reps were combined. bettercallsal detected all serovars in flour up to Day 56. Mbandaka was not identified after day 56 at 60%RH. At day 168, only Agona and Enteritidis were identified in flour at 60% humidity. Both kmer and bettercallsal identified 4 serovars in pistachio to day 365, except Oranienberg which was identified at low abundance. Seqsero2 was unable to identify any of the serovars.

Significance: By combining metagenomics and precision analysis tools, we can identify multiple serovars of *Salmonella* in contaminated foods. This study is of high importance for the development of culture-independent methods.

P3-181 Peptide Structures on Cecal Microbiota Inoculated with *Campylobacter jejuni*

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❖ ***Developing Scientist Entrant***

Introduction: One contributing factor associated with *Campylobacter* prevalence in poultry may be amino acid availability in poultry's gut.

Purpose: Characterizing microbiota involved in cross-feeding with *Campylobacter* may help to identify metabolic interactions and offer non-*Campylobacter* bacterial targets for interventions that serve to "starve" *Campylobacter*.

Methods: Effects of peptide supplementations of 3°, 2°, and 1° on microbial diversity were analyzed using an *in vitro* poultry cecal model inoculated with *C. jejuni*. Treatments included 3 peptide structures, a positive control (PC) inoculated with *C. jejuni* and a negative control (NC). Both controls did not include the supplemented peptides. The samples were collected at three time points: 0, 24, and 48h incubation under microaerophilic conditions at 42°C (N=90, n=6, k=5, time=3). Genomic DNA was extracted, amplified, and sequenced on an Illumina MiSeq. Sequencing data were analyzed in QIIME2-2021.11, with alpha and beta diversity determined using Kruskal-Wallis and ANOSIM.

Results: Microbial compositional diversity for supplemented groups was improved based on alpha diversity metrics ($P < 0.05$). Based on beta diversity, 2° and 1° produced significantly different relative abundances and phylogenies compared to PC and NC ($P < 0.05$). ANCOM differentiated *Campylobacter* as significantly different taxa in relative abundance between the treatments, with the highest relative abundance in 2° group. Based on core microbial analysis, only a 2° peptide structure supported *Campylobacter* core abundance (detection limit: > 5% in > 50% of samples) in 48h. Whereas *Faecalibacterium* was absent as a core member in 2° group in 24 and 48h.

Significance: *C. jejuni* displayed a molecular preference for 2° peptides over 3° and 1°, producing antagonistic interactions with probiotic bacteria such as *Faecalibacetrium*. Further metabolic profiling will facilitate an understanding of cecal ecology's function when supplemented with peptides and inoculated with C. *jejuni and* expand on cross-feeding relationships between *Campylobacter* and probiotic bacteria.

P3-182 Genomic Characterization of Probiotic *Bacillus* Strains for Poultry through Whole Genome Sequencing

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Introduction: Several *Bacillus* strains from sourdough and the gastrointestinal tract of broilers have shown potential as probiotic for poultry based on biochemical assays and animal feeding trials. Genomic characterization of these strains may provide further insights on their potential and working mechanisms as probiotics.

Purpose: This study aimed to characterize four potential probiotic *Bacillus* strains at genomic level through whole genome sequencing (WGS) and analysis.

Methods: Genomic DNA was extracted from pure culture of each strain using DNeasy blood and tissue kit. Whole genome shot gun sequencing was performed by Illumina NextSeq 500 with DNA libraries prepared using the Nextera XTDNA kit. Raw reads were trimmed, and quality checked by Trimmonatic and FastQC, respectively. De novo assembly was performed using SPAdes v.3.15.0. Genome annotation was done using Prokka v.1.14.6. Additionally, antibiotic resistant profiles were predicted using RGI v.6.0.0, and the detection of secondary metabolite biosynthetic gene clusters and bacteriocins was performed using antiSMASH v.6.1 and Bagel4, respectively.

Results: Only one antibiotic resistant gene *tet* (45) (resistance to tetracycline) was detected in two of the four strains studied whereas additional resistance genes to aminoglycosides (*ykkD*, *ykkC*, *aadK*) and macrolides (*vmlR*, *mphK*) were detected in the other two strains. An array of genes encoding bacteriocins such as amylocyclicin, subtilosin were detected in these strains.

Significance: This study demonstrated that genomic level characterization of probiotic bacteria can provide significant information about their mechanisms and safety in their industry application. The strains with fewer antibiotic resistance genes could be better choice in such application.

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P3-183 *Bacillus cereus* Enterotoxin Producers Induced Accelerated Bioenergetic Metabolism of Intestinal Caco-2 Cell Line

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❖ ***Developing Scientist Entrant***

Introduction: Bacillus cereus *sensu lato* is a Gram-positive, spore-forming group of bacteria composed of a growing list of novel species recognized as significant in medicine, food safety, and agriculture. To induce toxicoinfection, pathogenic enterotoxin producing *B. cereus* strains have to survive the hostile gastric conditions, reach the small intestine, and damage epithelial cells by pore formation. However, how pathogenic *B. cereus* rewires the metabolism of the intestinal cells is poorly characterized.

Purpose: The aim was this study was to assess metabolic changes in intestinal cells after infection with *B. cereus*.

Methods: Here, we used extracellular Seahorse XFe/XF analyzers to simultaneously measure oxygen consumption rates (OCR) and glycolytic (ECAR) in real time to estimate metabolic properties (energy phenotype, ATP production, and glycolytic proton rate) of human colorectal adenocarcinoma cell line (Caco-2) after exposure to three *B. cereus* pathotypes (NVH0075/95, NVH0391-98, ATCC 14579).

Results: *B. cereus* infection accelerated OCR and ECAR, indicating that Caco-2 cells switched from a quiescent state to a more energetic phenotype in response to infection. Strain ATCC 14579 caused the most pronounced effects towards specific energy phenotype (258.7 ± 21.1 pmol/min), followed by strains NVH0391-98 (227.8 ± 16.6 pmol/min) and NVH0075/95 (220.2 ± 29.3 pmol/min) in comparison with untreated control (189.5 ± 39.7 pmol/min). Also, Caco-2 cells manifested an increased ATP production rate (glycolytic and mitochondrial) after infection. Strain NVH0391-98 caused the highest ATP production rate (1648.9 ± 46.3 pmol/min), followed by ATCC 14579 (1551.37 ± 50.5 pmol/min) and NVH0075/95 (1441.6± 140.4 pmol/min). Although infected cells significantly increased glycolytic ATP production rate, mitochondrial ATP was the primary energy source, indicating oxidative phosphorylation as the main metabolic pathway and mitochondria as the leading energy producer.

Significance: Our findings enhanced the understanding of how intestinal cells can adjust their metabolism and successfully cope with *B. cereus* infection.

P3-184 Btyperdb: A Curated Public Database of *Bacillus cereus* Group Genomes and Metadata

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Introduction: The number of publicly available *Bacillus cereus* group genomes has grown rapidly; however, public databases currently housing *B. cereus* group genomes are plagued by taxonomic misclassifications, unstandardized metadata, and low-quality genomes.

Purpose: We developed BTyperDB (www.btyper.app), a database of publicly available and newly sequenced *B. cereus* group genomes and metadata.

Methods: All genomes submitted to the National Center for Biotechnology Information (NCBI) Genbank database as a *B. cereus* group species were downloaded (*n* = 3,428 genomes; accessed 8 August 2022). Sequence Read Archive (SRA) data of *B. cereus* group members with no linked assembly, as well as 75 newly sequenced *B. cereus* group genomes generated in this study, were assembled into contigs using Shovill v1.1.0/SKESA v2.4.0 or Unicycler v0.5.0 (*n* = 3,473 newly assembled genomes). All 6,901 genomes underwent quality control (via QUAST v5.0.2 and CheckM v1.1.3), taxonomic assignment (via BTyper3 v3.3.3 and the Genome Taxonomy Database Toolkit v2.1.0), and *in silico* typing (via BTyper3). Low-quality and misidentified genomes were removed, and the BTyperDB web application was implemented using React and Flask.

Results: BTyperDB nearly doubles the number of publicly available assembled *B. cereus* group genomes (*n* = 5,976 total high-quality genomes, compared to 3,428 assembled genomes in NCBI's Genbank Assembly database). Genomes originated from strains from 90 countries across six continents, plus two oceans and the International Space Station. Cereulide (emetic toxin) synthetase gene-harboring genomes, which have the potential to cause emetic foodborne illness, primarily belonged to sequence type 26 (138/173 genomes, 79.8%). However, 25 potentially emetic genomes resembled *B. mycoides*/*B. weihenstephanensis*; 20 of these genomes (80%) were assembled in this study.

Significance: BTyperDB is a free, curated, publicly available database of high-quality *B. cereus* group genomes with consistent taxonomic annotations and standardized metadata, which can help improve *B. cereus* group surveillance, source tracking, and outbreak detection efforts.

P3-185 An Exposure Assessment of Cytotoxic *Bacillus cereus* Strains from Various Phylogenetic Groups in HTST Milk

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❖ ***Developing Scientist Entrant***

Introduction: Food safety risks associated with *Bacillus cereus* strains from different phylogenetic groups vary depending on their cytotoxicity and growth capacity. Current guidelines for the maximum *B. cereus* levels in food differ but are 10^5 CFU/g or mL in some countries.

Purpose: The goal of this project was to model the growth of cytotoxic *B. cereus* strains from different phylogenetic groups in contaminated HTST milks along a supply chain, and to predict the *B. cereus* concentrations upon human consumption ("exposure assessment").

Methods: Growth data for 15 *B. cereus* strains (representing five *panC* groups) that showed growth in skim milk broth at 10°C and 22°C were fitted in Baranyi model to estimate the growth parameters. A reduced Ratkowsky model was selected as the secondary model. Time and temperature profiles from processing plant to consumer storage were simulated for hypothetical lots of HTST milk (100 iterations representing 100 half gallon units per lot), with separate simulations for each of 15 strains (with initial contamination of 100 CFU/mL). Final concentrations were predicted using the R package "biogrowth" with modifications.

Results: Our model predicted that 4.33 ± 2.47 (mean ± standard deviation) percent of the HTST milk had concentrations of *B. cereus* that exceeded the established maximum level of 10^5 CFU/mL on day 35 of home storage. Isolate PS00413, a *Bacillus cereus* s.s. strain from group IV and Isolate PS00638, a *Bacillus toyonensis* strain from group V grew faster than the other strains. Ten percent of the HTST milk contaminated with either strain was predicted to have exceeded concentrations of 10^5 CFU/mL on home storage day 35.

Significance: This model presents a tool that can help the industry to assess potential human exposure if HTST milk is found to be contaminated with a given *B. cereus* clade at a given level, facilitating management decisions.

P3-186 Comparative Genomic Analyses of Human- and Non-Human-Associated Isolates of *Salmonella enterica* Serotype Dublin

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❖ ***Developing Scientist Entrant***

Introduction: *Salmonella enterica* subspecies *enterica* serotype (*S.*) Dublin is commonly associated with cattle and can cause infections in humans.

Purpose: Iden*tify clades within S. D*ublin with an overrepresentation of human isolates and the associated genomic features that putatively contribute to an enhanced human virulence.

Methods: A maximum likelihood phylogeny was reconstructed from core SNPs for representative isolates of all *S.* Dublin SNP clusters and singletons available in NCBI Pathogen Detection. Subsequently, a subset of isolates from the largest clade with well-documented isolation time and sources were used to reconstruct a time-scaled phylogeny, based on which isolates were partitioned into human-associated (HA) and non-human-associated (NHA) groups. Selected genomes representing each group were annotated and used to conduct pan-genome analysis and pan-genome wide association studies.

Results: S. Dublin is monophyletic with four major clades (>100 isolates) identified. The largest clade containing 2,933 isolates was cattle-associated with a sub-clade showing an overrepresentation of human isolates. The pan-genome estimated for representative isolates of HA and NHA groups comprised 5,242 genes, wherein 4,205, and 1,037 were classified as core and accessory genes, respectively. Further comparative genomics identified 132 and 203 genes that were overrepresented in the HA and NHA groups, respectively.

Significance: Genes identified by this study can be selected as candidates in phenotypic experimentations to identify genomic characteristics associated with enhanced human virulence in *S.* Dublin. The findings of this study can facilitate understanding of the underlying mechanism of differential human virulence among isolates from *S.* Dublin, thereby improving control strategies targeting this important pathogen.

P3-187 Comparison of Genetic Characteristics of Six Different *Listeria* Species: *L. monocytogenes, L. innocua, L. welshimeri, L. grayi, L. aquatica*, and *L. fleischmannii* Isolated from Foods, Patients, and Farms

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* is a foodborne pathogen found in foods, patients, and environments. Pathogenic *L. monocytogenes* and non-pathogenic *Listeria* species including *L. innocua*, *L. welshimeri*, *L. aquatica, L. fleischmannii*, and *L. grayi* are known to share their living environments, suggesting

both may have the similar genetic characteristics make difficult to discriminate each other. In addition, the genetic features of non-pathogenic *Listeria* species have not been fully studied.

Purpose: The purpose of this study is to compare and identify the genetic features of six different *Listeria* species using a whole-genome sequencing (WGS).

Methods: A total of 18 *Listeria* strains were selected including four *L. monocytogenes*, six *L. innocua*, three *L. welshimeri*, two of each *L. aquatica* and *L. fleischmannii*, and one *L. grayi*. The WGS library was prepared using a Nextera XT DNA library kit and sequenced using an Illumina-MiSeq. Different genetic parameters of *Listeria* species were investigated based on multilocus sequencing typing (MLST), pangenome, functionality, virulence, and antibiotic resistance gene prevalence.

Results: The pangenome of 18 *Listeria* strains represented a total of 4,304 genes which included core genes (3,769), accessory genes (370), and unique genes (165). *Listeria innocua* genome showed more functionality in carbohydrate transport and metabolism while *L. fleischmannii* genome possessed relatively higher number of functional genes associated with the translation, ribosomal structure, and biogenesis. Each *L. innocua* and *L. welshimeri* possessed an average of 33 virulence genes and antibiotic resistance genes (*lin* and *fosX*). Three *L. monocytogenes* only possessed the *mprf* resistance gene, which disrupts the cell membrane defense peptides.

Significance: This study showed that non-pathogenic *L. innocua* and *L. welshimeri* have virulence and antibiotic-resistance genes. Understanding the genetic characteristics of non-pathogenic *Listeria* species is important to distinguish pathogenic *L. monocytogenes*.

P3-188 Comparison of Whole Transcriptomes of Stress-Resistant *Listeria monocytogenes* in Stress and Normal Growth Conditions

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Introduction: *Listeria monocytogenes* is a notable foodborne pathogen that can survive in stress growth conditions such as low pH, low temperature, and high salt concentrations. Although many studies on the resistance of *L. monocytogenes* were reported, entire gene expressions for survival under stress growth conditions have not been fully studied. We hypothesize that genes of stress-resistant *L. monocytogenes* will be differently expressed under stress environments.

Purpose: The purpose of this study is to compare transcriptomes of stress-resistant *L. monocytogenes* under stress and normal growth conditions.

Methods: The death curves of three stress-resistant *L. monocytogenes* strains were measured during 48 h of incubation in each stress (pH 3, 5% of NaCl, and 1°C) and normal growth condition (37°C) in tryptic soy broth (TSB). Total RNA of each strain was extracted at 0, 4, 8, 12, 24, and 48 h using a RNeasy micro kit (Qiagen). The Illumina stranded total RNA prep kit was used for the library preparation and whole transcriptomes were sequenced using an Illumina NextSeq2000. Raw sequencing reads were mapped using a STAR 2.15 tool and different gene expression analysis was conducted using a DESeq2 software.

Results: After 24 h incubation, three stress-resistant *L. monocytogenes* strains grown under the stress condition exhibited less survival than those grown under the normal condition. The average number of reads of the whole transcriptome was 5 million. A total of 43 genes including *ssrA* gene (tmRNA) under the stress condition were highly expressed than other genes.

Significance: This is the first study to examine the entire gene expressions of stress-resistant *L. monocytogenes* under the stress growth condition. Understanding the differently expressed genes of stress-resistant *L. monocytogenes* will contribute to identify the survival mechanisms of *L. monocytogenes*.

P3-189 Genetic Diversity of *Listeria monocytogenes* Collected from Ice Cream Production Facilities in the United States during 2016 and 2017

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❖ ***Developing Scientist Entrant***

Introduction: Between 2016 and 2017, environmental samples from 89 ice cream production facilities were collected and analyzed for the presence of *Listeria monocytogenes* (*Lm*) by the FDA. Overall, *Lm* was detected in 19 facilities (21.3%), and 65 of the 5,295 (1.25%) samples when samples from all facilities were combined.

Purpose: Whole-genome sequencing (WGS) data of *Lm* isolates recovered from the ice cream production facilities were analyzed to determine the genetic diversity of *Lm.*

Methods: WGS data of 65 *Lm* isolates recovered from 19 ice cream production facilities were retrieved from the GenomeTrakr database. Raw data were assembled using Qiagen CLC Genomic Workbench and molecular subtyping was performed using a core genome multilocus sequence typing (cgMLST) scheme. Based on the cgMLST profiles including 1,827 targets, the duplicate isolates from the same sample were determined and removed. *In silico* MLST and PCR-serogroups were performed to determine clonal complexes (CCs) and serogroups. The phylogenetic lineages were determined according to the phylogeny. The presence of major virulence and stress response genes were determined among the isolates.

Results: A total of 33 *Lm* genomes were selected after removing duplicates based on the 1,827-cgMLST profiles, 22 belonging to lineage I (67%) and 11 belonging to lineage II (33%). A total of 12 clonal complexes were identified with CC5 being the most predominant (49%) followed by CC155 (12.1%) and CC321 (9.1%). *Listeria* Pathogenicity islands (LIPI)-3 was found in four isolates (12%) and LIPI-4 was found in one isolate (3%). The full length of *inlA* gene, which contributes to bacterial invasion, was found in 25 isolates (76%). Plasmids were found in 28 isolates (85%), carrying the major genes contributing to multiple stress response in the environment.

Significance: Investigation on the genetic diversity of *Lm* isolates could help us to have a better understanding on its population structure in production environment of ice cream.

P3-190 Whole Genome Sequencing Analysis of an *Mcr-1*-Positive and Multidrug-Resistant *Escherichia coli* Isolated from Retail Chicken Meat in Lebanon

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❖ ***Developing Scientist Entrant***

Introduction: The reliance on colistin, a last resort antibiotic, in human medicine and agriculture has led to the dissemination of the plasmid-borne mobile colistin resistance genes (*mcr*) in Lebanon.

Purpose: Here, we used whole genome sequencing (WGS) to investigate the properties of an *mcr-1*-positive and multidrug-resistant *Escherichia coli* isolated from retail chicken meat in Lebanon.

Methods: Twenty-nine retail chicken samples were aseptically collected from grocery stores in Beirut (the capital of Lebanon). The samples were homogenized in buffered peptone water (BPW) and an aliquot (100 µL) was spread on RAPID*E.coli*2 agar supplemented with 4 µg/ml of colistin. Putative

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colistin-resistant *E. coli* were screened for (*mcr-1* to *mcr-8*) and other antibiotic-resistance genes using PCR. The disk diffusion and broth micro-dilution assays were used to determine the antibiotic-resistant phenotypes. WGS analysis was conducted to identify the resistome, virulome, and plasmid types.

Results: Thirty-four colonies, isolated from 17 chicken samples, yielded *mcr-1* signal using PCR analysis. Therefore, *mcr-1* was detected in 58% of the meat samples. The prevalence of *mcr-1* in chicken meat samples was significantly lower ($p < 0.05$) than that reported in the feces of preharvest chickens. One *mcr-1*-positive *E. coli* was identified using a gene- and species-specific PCR analysis. The isolate was multidrug-resistant, exhibiting resistance to 8 classes of antibiotics and a colistin MIC of 32 µg/ml. WGS analysis confirmed that the isolate harbored *mcr-1.26* and carried 28 additional acquired resistance genes, which were detected using ResFinder v.4.1. The isolate belonged to sequence type (ST3107) and carried various plasmid types, including IncX4, IncHI2, and IncHI2A. Analysis using Virulence Finder v.2.0 showed that the isolate harbored 8 virulence genes. *mcr-1.26* was successfully transferred to naïve JM-109 *E. coli* using the heat-shock assay, confirming that the gene was plasmid-borne.

Significance: The detection of transmissible *mcr-1.26* in multidrug-resistant *E. coli* in chicken meat highlights the risk of the dissemination of critically-important antibiotic resistance determinants in potential pathogens from farm to fork in Lebanon.

P3-191 Imported Seafood as a Reservoir of the Mobile Colistin Resistant Gene, *Mcr-9.1,* in the USA

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❖ ***Developing Scientist Entrant***

Introduction: The majority (65-85%) of the seafood in the U.S. retail market is imported. Notably, food imports, including seafood, have an established role in disseminating important antibiotic resistance (ABR) genes across geographic borders. However, the role of seafood in the introduction of the mobile colistin resistance genes (*mcr*) to the USA has not been investigated. This is especially important, because colistin, a last-resort antibiotic, is used to treat recalcitrant infections.

Purpose: Here, we assessed the role of imported seafood as a potential reservoir of *mcr* in the USA.

Methods: Imported seafood samples (n=45) were collected from retail stores in Georgia, USA. Samples were homogenized in buffered peptone water (BPW) and plated on the chromogenic RAPID' *E. coli*2 agar supplemented with 4 µg/ml of colistin. The agar is commonly used to isolate *E. coli* and other fecal coliforms from foods and other matrices. Putative colistin-resistant colonies were screened for (*mcr-1* to *mcr-10*) by PCR. Disk diffusion and broth microdilution assays were used to assess the antibiotic-resistant profile of the isolates against 19 clinically- and agriculturally- important antibiotics. Isolates with high colistin minimum inhibitory concentration were subjected to Whole-genome sequencing (WGS). Transformation (heat-shock) and biofilm assays were used to determine if the detected *mcr* were plasmid-borne and affected the fitness of the isolates.

Results: ***mcr-9.1*** was detected in *Serratia nevei* (n=5) from imported shrimp samples. The isolates were highly resistant to colistin (MIC> 640 µg/ml) and were also resistant to other important antibiotics (tetracycline and streptomycin). WGS analysis showed that the isolates harbored additional acquired antibiotic-resistance genes (*aac(6')-Ic*; *tet(41)*; bla_{SRT-2}). Various plasmid types were detected in the isolates, including IncF and IncHI2. *mcr-9* was successfully transferred to naive *E. coli* DH5α cells and was found to be carried on an IncHI2 plasmid. Furthermore, *mcr-9* persisted in 12-day-old biofilms.

Significance: Seafood imports might play an important role in introducing *mcr* and other antibiotic-resistant determinants to the food supply chain in the USA.

P3-192 Identification of New O-Antigen Gene Clusters and Development of Multiplex PCR for O-Antigen Classification in *Escherichia coli*

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❖ ***Developing Scientist Entrant***

Introduction: *Escherichia coli* (*E. coli*) O-antigen serotyping plays a vital role in diagnosis, epidemiological studies, outbreaks investigations and public health monitoring.

Purpose: As whole genome sequencing is becoming more accessible, unknown *E. coli* strains that do not match the current designated O types are increasingly being recognized, creating a knowledge gap on the possible virulent threats these new strains may contain.

Methods: In this research we gathered a collection of *E. coli* isolates that were suspected to have new O-antigen gene clusters (O-AGCs). The isolates were sequenced using MinION nanopore sequencing and the O-AGCs were identified and annotated by using Prokka. BLAST comparisons between O-AGCs were generated and visualized with Easyfig. *Wzx/wzy* and *wzm/wzt* biomarker genes were identified, the sequence percent identity was analyzed, and primers for simplex and multiplex PCR were generated. Primer *in silico* validation was performed to check efficiency and specificity. Wet lab validation was performed by PCR amplification.

Results: Forty-nine *E. coli* isolates presenting new O-AGCs were successfully identified. The isolates come from a diverse group of samples: (39/49; 80%) terrestrial animals, (6/49; 12%) avian, and (4/49; 8%) environmental samples. The cluster of genes responsible for O-AGCs biosynthesis were located between the *galF* and *gnd* locus. BLAST sequence identity analyses revealed that all isolates contained unique O-AGCs and unique *wzx/wzy or wzm/wzt* biomarker genes. Simplex and multiplex PCR assays were successfully designed to amplify the target genes with distinct amplicon sizes for each O-AGC that can be readily identified within each assay.

Significance: Identifying *E. coli* isolates expressing new O-antigens gene clusters and the generation of primers for O-antigen classification for these newly identified strains, helps to refine current technologies of detection that can be used for epidemiological studies as well as for the surveillance of pathogenic *E. coli* to reduce the pathogen detection time in clinical cases and outbreak investigations.

P3-193 Detection of Norovirus Capsid Using Surface-Enhanced Raman Spectroscopy

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❖ ***Developing Scientist Entrant***

Introduction: Human noroviruses are the leading cause of foodborne illnesses in the US and globally. There are numerous norovirus detection techniques, however, many have limitations with regards to lack of portability, requirement for a high degree of required sample manipulation prior to detection, rapidity, and specificity. Compared to traditional methods, surface enhanced Raman spectroscopy (SERS) is considered a promising analytical method, capable of producing sensitive detection of target with fingerprint signals and minimal required manipulation of sample matrix prior to detection.

Purpose: The purpose of this study was to develop a SERS-based method to detect norovirus using affinity peptide labeled gold nanoparticles (AuNPs).

Methods: Two norovirus peptides previously reported to bind noroviruses, Noro-1 (QHKMHKPHKNTK) and NV-O-R5-3 (LDYRSWAPYATS), were expressed with *E. coli*, purified by nickel column, and labeled onto 50 nm AuNPs with the addition of glycine and serine linker. The peptide-labeled AuNPs were used as a SERS substrate and Raman measurements taken using a DXR Raman Spectro-microscope with 780 nm laser wavelength and 2 seconds of exposure, after incubation with 0.3-1 mM capsid protein.

Results: Compared to the Raman signal of substrate only, peptide Noro-1-labeled substrate did not generate observable unique signal after incubation with 300 nM of capsid protein for 20 minutes. NV-O-R5-3-labeled substrate showed a significant peak at 750 cm^{-1} after 20 minute incubation with 1uM of

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capsid protein meaning it has a capability to detect noroviruses. One uM BSA did not generate similar signals under the same conditions, suggesting that NV-O-R5-3-labeled substrate demonstrated specificity.

Significance: These data suggest that SERS shows potential for specific detection of noroviruses.

P3-194 Dynamic Fluctuation and Niche Differentiation of Fungal Pathogens Infecting Bell Pepper Plants

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Introduction: Plants accommodate numerous microbes, including bacteria, fungi, archaea, protists, and viruses. Fungal pathogens can colonize in the niches of the host plants and infect fruits, leaves, stems, and roots of crop plants at both pre-and postharvest stages, leading to a reduction of crop yield and economic loss. Dynamic fluctuation of the microbiome and fungal pathogens in bell pepper plants is poorly understood, and the origin of fungal pathogens causing fruit rot and leaf wilt has been barely investigated.

Purpose: This study explored the compositional variations of the microbiome in bell pepper plants and studied the fluctuation of fungal pathogens across the growing seasons.

Methods: In this study, we used amplicon sequencing (i.e., 16S rRNA and internal transcribed spacer [ITS] sequencing) to explore the compositional variations of the microbiome in bell pepper plants. Co-occurrence network analysis was applied to track fungal pathogens' origin and dissemination route that infected bell pepper plants.

Results: ITS and 16S rRNA sequencing analyses demonstrated that fungal pathogens infecting fruits and leaves probably belonged to the *Penicillium*, *Cladosporium*, *Fusarium*, and unclassified_Sclerotiniaceae genera rather than one specific genus. Fungal pathogens decreased species richness and diversity of fungal communities in fungus-infected fruit and leaf tissues but not the uninfected stem tissues. Bacterial metabolic functions of xenobiotics increased in fungus-infected leaves at a mature developmental stage. Competitive interaction was present between fungal and bacterial communities in leaves. Co-occurrence network analysis revealed that the origins of fungal pathogens included the greenhouse, packing house, and storage room. Niche differentiation of microbes was discovered among these locations.

Significance: This study improves the understanding of dynamic fluctuation and the source of fungal pathogens infecting bell pepper plants in the farming system. The findings facilitate the precise management of fungal pathogens in the greenhouse.

P3-195 Investigation of an Artisanal Cheese Manufacturing Defect by Next Generation Sequencing

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Introduction: Natural Whey Starter (NWS) culture is obtained by the backslopping method from the process of artisanal cheesemaking and exhibit a complex microbiota that affects physico-chemical properties and the quality of cheese. The microbial composition of the NWS may cause defects in cheese that leads to financial and image damage to producers.

Purpose: This study investigated the microbiome of two artisanal cheeses from Serra da Canastra, Brazil, made with different NWS, one that produced a cheese with a defect ("swelling cheese"), and the other that produced a cheese of good quality.

Methods: Cheeses were made from raw milk and ripened for 21 days at 20 °C and 65% humidity. Microbiome of the two NWS, "swelling" and "good", and respective cheeses were evaluated by next generation sequencing. The trials were repeated twice, and samples (n=54) were analyzed in triplicate.

Results: Swelling defect appeared around the seventh day of ripening. Alpha diversity was different among the two NWSs ($P<0.05$). Genera prevalent in "swelling" NWS were *Lactococcus* and *Escherichia* while in "good" NWS were *Streptococcus*, *Staphylococcus* and *Lactobacillus*. Samples showed a significant difference ($P<0.05$) in microbiota composition between the two batches at each sampling time (0, 7, 14 and 21 days) using the PERMANOVA test. It was possible to observe an increase in the abundance of *Escherichia* in "swelling" cheese on the seventh day. These results suggest that the swelling defect is due to *Escherichia* and it is possible that the absence of *Streptococcus* and the higher proportion of *Lactococcus* in "swelling" NWS did not inhibit *Escherichia*.

Significance: This approach allowed the probable identification of the causative microorganism of swelling in artisanal cheeses. Further studies of the microbiome of NWS could help to prevent the appearance of swelling defect, increasing the value of the product.

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P3-196 Microbial Diversity of Selected Ripened Cheese Varieties Produced in Uganda

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Introduction: Production of cheese of consistent quality requires knowledge of its dominant microflora that generates desirable quality attributes.

Purpose: This study determined diversity of microorganisms in ripened cheese varieties produced in Uganda in order to evaluate their contribution to desirable cheese qualities.

Methods: Different brands (n=12) of ripened (1-2 months) cheese were purchased from local supermarkets. Three ripened (3 months) Gouda cheese varieties from Netherlands were included as reference samples. All samples were delivered to Makerere University Microbiology laboratory under ice, and analyzed within 8 h. Each sample (25 g) was diluted in 225 mL of peptone water (PW) to obtain a dilution factor of 10^{-1}. Further dilutions up to 10^{-7} were prepared in PW. All diluted samples were plated in triplicate on MRS, M17, potato dextrose and nutrient agar, and incubated aerobically for 1-5 days at 25-37°C. Colonies were enumerated and results normalized by conversion into log CFU/g and analyzed using one-way ANOVA with post-hoc Tukey HSD test ($p<0.05$). Isolates were recovered from different plates by streaking twice on respective media, and examined for Gram reaction, catalase & oxidase tests, cell morphologies and biochemical characteristics in order to determine their presumptive identities.

Results: Cheese was dominated by lactic acid bacteria (6.82±0.20 log CFU/g) and yeast & molds (2.54±0.05 log CFU/g). None of the thermotolerant coliforms, *Staphylococcus* spp. and *Escherichia coli* were detected (LOD, 1 log CFU/g). The presumptive microbial genera (n=78 isolates) were; Gram positive, catalase and oxidase negative cocci (89.3%, *Streptococcus*, *Enterococcus* or *Lactococcus* spp.), and Gram positive, catalase and oxidase negative rods (10.7%, *Lactobacillus* spp.).

Significance: LAB in ripened cheese produced in Uganda belong to diverse genera. LAB enhance quality properties of fermented foods. The isolates are undergoing speciation using PCR, to enable us probe for their technological properties.

P3-197 Genomic Analysis of *Salmonella* with Decreased Susceptibility to Azithromycin Isolated from Food Animals and Retail Meats in the U.S.

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Introduction: Azithromycin, a 15-membered ring macrolide antibiotic, is critically important to human medicine and can be used to treat *Salmonella* infections. Other macrolides with 14-, 15-, and 16-membered rings are commonly used in veterinary medicine.

Purpose: We aimed to study the genomic structure of decreased susceptibility to azithromycin (DSA) in *Salmonella* recovered from food animals and retail meats and assess the contribution of macrolide resistance genes to minimum inhibitory concentration (MIC) changes for macrolides with different ring structures.

Methods: Thirty-seven *Salmonella* isolated in 2015–2021 that either showed DSA (MIC ≥32 μg/mL) or contained macrolide resistance genes were identified through the National Antimicrobial Resistance Monitoring System. Antimicrobial susceptibility testing (AST) was performed using broth microdilution; MiSeq and PacBio assemblies were analyzed with AMRFinder-plus and PlasmidFinder.

Results: Resistance mechanisms identified included *ere*(A), *erm*(42), *erm*(B), *mef*(C), *mef*(B), *mph*(A), *mph*(E), *mph*(G), and *msr*(E), and a point mutation (*acrB*_R717L). Among these, *mph*(A) was dominant (56.8%). A macrolide custom AST panel showed that these genes accounted for up to 256-fold increases in MIC against 14- and 15-membered macrolides compared with *Salmonella* isolates that lack macrolide resistance genes. The *erm*(42) and *acrB*_R717L were associated with 4-128-fold higher MICs to the 16-membered macrolide tildipirosin. High MICs for most other 16-membered macrolides were observed in *Salmonella* with and without the macrolide resistance genes. Macrolide resistance genes were mapped to diverse plasmid replicons, including Col(pHAD28), IncC, IncFIA, IncFIB(K), IncHI1A, IncHI1B, IncHI2, IncN, IncP6, IncQ1, and IncR. Some were hybrid mega-plasmids containing a wide range of genes conferring resistance to multiple drug classes, including critically important antimicrobials such as 3rd generation cephalosporins and fluoroquinolones.

Significance: Emergence of DSA *Salmonella* in food animals and derived meats with co-resistance to critically important antimicrobials is a major public health concern, which warrants continued monitoring and intervention strategies to combat such resistance.

P3-198 Evaluation of Romaine Lettuce Quality and Microbial Ecology uder Source Processing and Forward Processing Conditions

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Introduction: Fresh-cut romaine lettuce has been implicated in multiple outbreaks of *Escherichia coli* O157:H7 in recent years. Traceback investigations potentially implicated regionally marketed fresh-cut products from forward processing plants.

Purpose: To assess the quality and microbiome dynamics of forward- and source-processed romaine lettuce products from harvest to storage.

Methods: Freshly harvested romaine lettuce from a commercial field lot destined to both forward and source processing facilities was tracked from farm to processing facilities by embedding data loggers in designated bins. Whole romaine lettuce heads from the farm and the tracked bins delivered to forward and source facilities before processing, and packaged fresh-cut lettuce after processing, were collected for quality and microbiological analyses, including total bacteria, coliform, yeast and mold counts (6 replicates). Both forward- and source-processed romaine lettuce samples were stored at 5°C and tested 1, 4, 7, and 14 days after processing (6 replicates). 16S rRNA high-throughput sequencing was also performed to determine the shift in bacterial communities under these processing conditions.

Results: For both forward and source processing, the harvested romaine lettuce was maintained at a temperature below 5°C and humidity above 90% during delivery and inventory storage before processing. A noticeable decrease (up to 20 kPa) of air pressure during forward delivery was recorded. Considering product quality, the loss of tissue integrity of forward-processed romaine lettuce, especially after 2-week storage, was significantly higher than source-processed products ($p<0.05$). Populations of microbial counts, especially total bacteria and coliform, of both whole-head lettuce before processing and fresh-cut lettuce after storage from forward facilities were significantly higher than that from source facilities ($p<0.05$). Microbiome analysis indicated that romaine lettuce production season, postharvest delivery, and processing could significantly affect lettuce microbiota and microbial ecology.

Significance: Data derived from this study provide insights into the development of preventive controls on *Escherichia coli* O157:H7 contamination during romaine lettuce production.

P3-199 *Salmonella* Contamination and Microbial Dynamics of Diced Tomatoes during Washing and Storage as Affected by Sanitation Treatments

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Introduction: *Salmonella enterica* is a major foodborne pathogen associated with consumption of fresh tomatoes, and *Salmonella* contamination during post-harvest handling has been suspected and implicated in multiple food-borne illness outbreaks.

Purpose: To examine the shift in *Salmonella* and indigenous microbiota on diced tomatoes after washing with different sanitizers and during storage.

Methods: Roma tomatoes were inoculated with a *Salmonella* cocktail (initial level ~ 5.6 CFU g^{-1}), diced and washed along with uninoculated diced tomato in simulated flume wash water with sanitizers, including 10 mg L^{-1} free chlorine (FC10), 90 mg L^{-1} peracetic acid (PA90), PA90 in combination with a proprietary acidified surfactant blend (PS90), and unsanitized control (CK). *Salmonella*, total bacteria, and yeast and mold (YM) populations on both inoculated and non-inoculated samples were measured before, after washing and during storage at 4 °C (3 replicates per sample). 16S rRNA high-throughput sequencing was also performed to determine shift in bacterial microbiome. ANOVA, Chi-square, Kruskal–Wallis H test and PERMANOVA were performed for statistical analyses of microbial and sequencing data.

Results: Washing with all tested sanitizers, especially PS90, efficiently reduced *Salmonella* and bacterial populations on inoculated diced tomatoes after washing. Application of sanitizers significantly mitigated *Salmonella* cross-contamination on non-inoculated samples. PS90 treatment inhibited the proliferation of most dominant bacteria on diced tomatoes during storage, such as *Erwiniaceae*, *Curtobacterium*, *Pantoea*, *Erwinia* and Enterobacterales, which may benefit product quality and safety. YM populations were similar among washing treatments with sanitizers.

Significance: This study provides in-depth information about the microbial ecology of the diverse bacterial communities on diced tomatoes during washing and the subsequent storage, which provide clues for the mitigation of *Salmonella* contamination, and control of plant pathogens and spoilage bacteria.

Poster

P3-200 Characterization of Virulence and Metabolic Gene Functions within Prophage Regions of >200 *Salmonella enterica* Serovars

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❖ ***Developing Scientist Entrant***

Introduction: Prophage regions within bacterial genomes can carry virulence and metabolic genes, which may increase the *in vivo* fitness of the bacterial host.

Purpose: The overall objective of this study was to characterize prophage regions within *Salmonella enterica* subspecies *enterica* (*S. enterica*) isolates to understand their role in the acquisition of novel virulence and metabolic functions in *Salmonella.*

Methods: We analyzed the whole genome sequence data of 242 *S. enterica* isolates representing 217 different serovars. Intact, incomplete, and questionable prophage regions were quantified from output from Phaster. Presence/absence of prophage were mapped onto a core SNP phylogeny inferred with IQ-TREE and were visualized in iTOL. EggNOG was used to classify coding sequences into clusters of orthologous groups (COG) categories to allow for further classification and identification of potential virulence factors and metabolic-associated functions.

Results: On average, 3.87% (range: 3.52% - 4.87%) of the genomic content of each isolate was annotated as prophage regions; this was consistent across different phylogenetic clades (p-value = 0.978; ANOVA). Among 1,918 total prophage regions, 569 were considered intact. Gifsy 1 and Salmon Fels 1 were the two most common intact prophage identified, with none of the nine most common prophage being associated with a specific clade. Among the 37 unique COG categories, we identified virulence factors associated with membrane biogenesis, motility, and metal ion metabolism within intact prophage regions. These factors included proteins associated with O antigen conversion, type 1 fimbriae assembly, arsenical and copper resistance, and siderophore interactions.

Significance: Our study provides new information about the cargo that prophage carry, and the role that they may play in the transmission of virulence factors and novel metabolic functions that may facilitate expansion of *Salmonella* into a new niche.

P3-201 Survival and Expression of Acid Resistance Genes of *Escherichia coli* O157:H7 in the Stomach Contents of Cattle

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❖ ***Developing Scientist Entrant***

Introduction: Healthy cattle serve as a reservoir for *E. coli* O157:H7. Understanding the survival and adaptation of *E. coli* O157:H7 inside the ruminant stomach will help to develop risk mitigation strategies.

Purpose: This study evaluated survival and expression of acid-resistance genes of *E. col*i O157:H7 inside the stomach contents of cattle.

Methods: Stomach contents, Rumen (Ru), Reticulum (Re), Omasum (Om), and Abomasum (Ab) of 7 cattle slaughtered in a USDA-inspected facility were collected. The contents were evaluated for the detection of *E. coli* O157:H7 using real-time PCR. All stomach contents (pH of Abomasum adjusted to 3 and 2) were inoculated with *E. coli* O157:H7 and incubated in anaerobic condition at 39°C for 4h. Bacteria were quantified using RT-PCR and propidium monoazide (PMA) was applied to detect viable bacteria in Abomasum contents. Analysis of expression of acid-resistant genes (RpoS, adiA, and gadA) after exposure to low pH of abomasum content for 4h was determined with RT-PCR and 2^-ΔΔCT. All analysis were performed in triplicate and data was analyzed using ANOVA.

Results: *E. coli* O157:H7 was not detected in stomach content of any cattle. After inoculation and 4h of incubation, bacterial count increased significantly (P<0.05) in rumen (2 to 8 log), reticulum (1.4 to 3.3 log), and Omasum (0.8 to 2.8 log) of all cattle. Bacterial count decreased significantly (P<0.05) in abomasum contents (~1 log reduction) with pH 3 and 2 in four out of seven cattle. However, bacteria increased significantly (P<0.05) in abomasum samples of three cattle regardless of pH. RT-PCR showed increased gene expression of RpoS and gadA upon exposure to lower pH of abomasum contents. RpoS was induced >100-fold in five of seven abomasum samples with pH 2 and was upregulated in all seven samples with pH 3 and 2.

Significance: This study showed that *E. coli* O157:H7 develop resistance to higher acidity and is able to survive in low-pH of stomach contents of cattle.

P3-202 Assessing the Mutational Rates of Bacterial Foodborne Pathogens in Different Agricultural Environments during Long-Term Colonization or Environmental Cycling

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Introduction: Mutations can be a major driving force of bacterial adaptation. Therefore, it is important to quantitatively analyze mutational rates of bacterial foodborne pathogens that occur in different agricultural environments over a prolonged period or during cycling between environments.

Purpose: The purpose of this study was to determine the mutational rates of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in agricultural soil and irrigation water during long-term colonization or cycling between different environments.

Methods: Microcosms of either 10 g of soil, 100 ml irrigation water, or 100 ml buffered peptone water (control) were inoculated with ~10^9 CFU of one of the three pathogens and then cultured/sampled every 2 week for 42 weeks. Long-term colonization microcosms were inoculated only once at the beginning of the 42 weeks, whereas environmental cycling consisted of inoculating a new microcosm with the pathogen isolated from the previous time point microcosm across 42 weeks. Shotgun metagenomics sequencing (Illumina NovaSeq S4) was performed on all samples (n=572) resulting in an average of 1.2 gigabases of sequencing data/sample. Reads were quality trimmed based on Phred score (Q33) using Trim-galore, assembled using MetaSPades, and finally contigs and filtered reads used to identify mutations or single nucleotide polymorphisms (SNPs) using the Bowtie2 plugin in Geneious Prime (v2022.1) against the corresponding assembled inoculum genome for each time point of the study.

Results: No mutations were observed in any long-term microcosms. However, soil cycling resulted in mutational rates of up to 2.48 mutations/yr for *E. coli* O157:H7, up to 2.48 mutations/yr for *S.* Typhimurium, and up to 6.19 mutations/yr for *L. monocytogenes*, whereas water cycling had up to 1.24 mutations/yr for *E. coli* O157:H7, up to 2.48 mutations/yr for *S.* Typhimurium, and up to 3.71 mutations/yr for *L. monocytogenes*.

Significance: Our results provide critical insights into how these pathogens function and adapt to different agricultural environments.

P3-203 Transcriptomic Analysis of *Vibrio cholerae* Biofilm Formation after Citric Acid Exposure on Food Contact Surfaces.

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❖ ***Developing Scientist Entrant***

Introduction: Vibrio cholerae is a foodborne pathogen associated with the consumption of contaminated seafood causing a severe disease. The microorganism is capable to form biofilm on food-contact surfaces and persist in the food-processing environment becoming a contamination source. The exposure to factors such as organic acids used during food preparation might affect the biofilm formation.

Poster

Purpose: The purpose of this study was to evaluate the differential gene expression of planktonic and biofilm immerse citric acid exposed *V. cholerae* cells on plastic surfaces.

Methods: *V. cholerae* VC6 strain in logarithmic growth-phase cell were exposed to trypticase soy broth-2% NaCl (TSBN) acidified with citric acid (pH 5) per 60 min. Cells were inoculated on plastic surfaces, immersed in TSBN (pH 5), and incubated at 25°C/4 h. Surfaces were rinsed to remove non-attached cells and incubated at 25°C/97% relative humidity for 24 h. Non-stressed cells were used as a control. Planktonic stressed cells and biofilm immersed cells were subjected to RNA extraction and a total RNA sequencing was conducted using Illumina HiSeq 2500 system. Differences in gene expression were analyzed using edgeR, and P-values were adjusted using multiple hypothesis tests.

Results: From 1284 genes only 358 (27.9%) were differentially expressed (FDR<0.05). *V. cholerae* cells showed different gene expression profile in stressed and non-stressed planktonic cells and immerse into biofilms. The main differences consisted in the repression of key genes involved in traditional biopolymers production pathways and induction of novel/non-frequent biopolymers production pathways, and induction of some heat shock proteins, anti-disruption membrane proteins and membrane structural proteins; these changes negatively impact the amount, structure, and stability of the biofilm.

Significance: Changes of gene expression of *V. cholerae* cells subjected to acidic stress are associated to modifications in the structure and composition of biofilms which suggest a less stable biofilm formation on plastic food contact surfaces.

P3-204 Significance of the Processing Environment of Frozen Vegetables as a Source of Contamination of *L. monocytogenes*

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Introduction: Frozen vegetables, and particularly frozen corn, have been associated to a large European outbreak of *Listeria monocytogenes* serogroup IVb. The outbreak investigation identified the processing environment, and more specifically the freezing tunnel, as the source of contamination. This outbreak evidenced the need to understand which are the most relevant niches of contamination of *L. monocytogenes* and its suitability to persist in the freezing environment.

Purpose: To determine which are the most relevant niches of contamination from the environment of frozen vegetables processing plants and to establish the genetic relationships of *L. monocytogenes* isolates found in the processing environment as well as in frozen vegetables

Methods: A total of 78 sampling points were selected from the environment of the freezing plant. Samples were characterized in two zones: food-contact surfaces (FCS); and non-food-contact surfaces (n-FCS). Samples of raw material and final product were also analysed. The genetic relationships among the *L. monocytogenes* isolates were characterizing by whole genomic sequencing.

Results: Prevalence of *L. monocytogenes* in the processing environment was high *L. monocytogenes* was found in 24% of the n-FCS (24%) and in 13% of the FCS. However, only one sample of final product was found to be positive for *L. monocytogenes*. Whole-genome sequencing (WGS) analysis revealed the presence of four different sequences types (ST) being ST7 the most prevalent followed by ST5, ST87 and ST8. The isolate obtained from the final product was the same as one isolate found in n-FCS, indicating the relevant role that n-FCS can play in spreading *L. monocytogenes* contamination to the final product.

Significance: Based on the results obtained, current monitoring programmes implemented in the frozen industry should be revised and intensify to reduce potential source of contamination with *L. monocytogenes*, mostly because consumer are more and more wrongly considered frozen vegetables as a ready to eat food.

P3-205 Impacts of Growing Conditions and Diet on the Microbiome of Turkeys

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Introduction: Compared to other domestic livestock and poultry, the microbiome of turkeys has been far less extensively studied. Similarly, feed additives have been shown to exhibit beneficial effects in chickens and swine, but their effects in turkeys are less well characterized.

Purpose: This study addresses both of these knowledge gaps by leveraging the power of DNA metabarcoding to ascertain the microbiome of turkeys over the average flock cycle and potential impacts that modifications by the administration of a yeast-derived feed additive may have on that microbiome.

Methods: Two different farms each with an experimental and a control barn were sampled at three different time points per flock over the course of three flock cycles. At each sampling time point four boot swabs were collected from each barn. At harvest, cecal samples were collected from ten birds from each flock. A total of 144 boot swabs and 120 cecal samples were collected. DNA was extracted from each sample and used as template for 16S rDNA metabarcoding analysis.

Results: Distinct microbial communities were noted for each farm. Similarly, cecal populations were clearly distinguishable from environmental boot swab samples. Sampling time was also found to be a significant factor for the establishment of distinct microbial communities. In several instances, the inclusion of feed additives to the diet increased microbial diversity while making significant impacts on the turkey-associated microbiome.

Significance: Advances in next generation sequencing have enabled the study of entire bacterial populations, here those technologies provided insight into the cultivation of domestic turkey populations. The identification of predominant taxa at key life stages, and under varying conditions provided useful insights into the best practices for the rearing and maintenance of turkeys, as well as inform the most efficacious use of feed additives.

P3-206 Genomic Characterization of Yeasts Strains Isolated from Food-Production Environments

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Introduction: Food production facilities are harsh environments employing many control strategies to eliminate pathogens and other spoilage microorganisms. This can result in the selection for extremotolerant microorganisms that are capable of either surviving or even growing in such harsh conditions, effectively negating food preservation efforts. Gaining a deeper understanding of spoilage yeasts, including their genetics, will address knowledge gaps associated with spoilage microorganisms.

Purpose: This study sought to investigate the genomes of several yeast species, derived from food-production environments, to address these knowledge gaps as well as characterize food plant-associated adaptations potentially caused by such harsh environments.

Methods: Strains of common food spoilage yeasts, including three strains of *Candida parapsilosis* and three strains of *Zygosaccharomyces bailii*, were characterized by whole genome sequencing on the Illumina MiSeq platform. Resulting reads were quality scored with fastqc, assembled using SPAdes and genome completeness was assessed with BUSCO. Next, comparative analysis was carried out using the CFSAN-SNP pipeline, Pangloss, and ANNOVAR.

Results: MiSeq sequencing was found to be sufficient for achieving greater than 200X coverage for *C. parapsilosis*. Coverage for *Z. bailii* was not as robust but considering its much larger genome (20Mb vs. 13Mb for *C. parapsilosis*), an entire MiSeq sequencing run devoted to one strain is required to achieve adequate depth for this organism. Comparative analyses demonstrated that food plant-derived strains were unique from strains publicly available from NCBI, potentially harboring unique SNPs in genes conferring improved survival traits.

Significance: Spoilage organisms, despite having deleterious impacts on food quality and food availability remain poorly characterized, particularly yeasts which have larger and more complex genomes. Here we provide genomic characterization of common spoilage yeasts derived from food-production environments, which will better inform for improving mitigation and control strategies for dealing with these extremotolerant organisms.

P3-207 Transcriptomic Responses of Aflatoxin-Producing *Aspergillus flavus* to Atmospheric Cold Plasma Treatment

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Developing Scientist Entrant

Introduction: Although atmospheric cold plasma (ACP) has shown potential as a rapid decontamination technology in the control of Aflatoxin-producing *Aspergillus flavus* for the food and feed industries, the specific mechanisms and modes of action that result in fungal inactivation by ACP are not well understood.

Purpose: This study aimed to assess the transcriptomic responses of Aflatoxin-producing *A. flavus* (spores and mycelia) to ACP treatment to determine the possible inactivation mechanisms.

Methods: Total RNAs were extracted from samples of spores and mycelia (wet and dry) of Aflatoxin-producing *A. flavus*-after ACP treatment (untreated as controls) and sequenced using Illumina platform. Bioinformatic analyses of mRNA sequences were done by mapping to reference genome, gene expression quantification, differential gene expression analysis (a fold change at least 1.5 and *P* value ≤ 0.01). Pathway and functional analyses were done by GO and KEGG enrichment.

Results: The largest numbers of up- and down-regulated genes were observed in spores (2094 ups and 1790 downs), comparing to wet and mycelia (194 and 104 ups and 118 and 71 downs, respectively). Majority of the DEGs in spores are related to ribosome and ribosome biogenesis whereas those in mycelia are associated with carbon mentalism, fatty acid degradation, and peroxisome. Genes related to oxidative stress such as NADH oxidoreductase, cytochrome C reductases were up regulated.

Significance: This study demonstrated that whole genome wide transcriptomic analysis of the responses of Aflatoxin-producing *A. flavus* to ACP treatment can provide much more insights into the possible involvement of several core genes and pathways in ACP inactivation mechanism.

P3-208 Inactivation of Aflatoxin-Producing *Aspergillus flavus* by Atmospheric Cold Plasma

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Developing Scientist Entrant

Introduction: Due to their detrimental effect on human and animal health, mycotoxin and mycotoxin-producing fungal contamination of grains and nuts has been a longstanding concern for both the food and feed industries. Postharvest decontamination is one of the critical steps in mitigating the problem. Atmospheric cold plasma (ACP), a new decontamination technology, has the potential to be employed by the industries to eliminate mycotoxin-producing fungi on the grains and nuts.

Purpose: This study aimed to investigate the inactivation efficiency of Aflatoxin-producing *Aspergillus flavus* (spores and mycelium) by ACP device using surface dielectric barrier discharge (SDBD).

Methods: Spores of *A. flavus* deposited on coverslips were treated with ACP at distance of 2/5 cm for 5 minutes and the survival spores were recovered and enumerated, with untreated spores as controls. Plugs of mycelia of *A. flavus* grown on potato dextrose agar plate were treated with ACP at 2/5cm for 5 minutes. Growth of mycelia (treated and untreated) were monitored for 5 days. Data collected from at least two separate trials with three replicates per treatment in each trial was used in data analysis with both One Way ANOVA and Student T-test. Additionally, scanning electron microcopy (SEM) was used to visualize the effect of ACP treatment on spores and mycelia.

Results: Inactivation of spores was observed at both distances but with increasing efficiency at shorter distance. An average 3.8±0.4 log reduction of spores was achieved at 5 min ACP treatment at 2 cm distance. Mycelia re-growth was significantly ($P<0.05$) inhibited after ACP treatment. SEM revealed visible shrinkage of mycelia but minimal physical surface damage on spores after ACP treatment.

Significance: This study demonstrated that SDBD-based ACP technology can be applied in the control of Aflatoxin-producing *Aspergillus flavus* (both spores and mycelia) in food to safeguard the health of human and animals.

P3-209 Transcriptional Response of *Salmonella enterica* after Bacteriophage Treatment

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Developing Scientist Entrant

Introduction: Salmonella enterica (*S. enterica*) is a causative agent of numerous foodborne outbreaks. Current industrial antimicrobial interventions have shown microbial reductions by <90%, allowing populations to survive and cause outbreaks. Consequently, bacteriophages (phages) have been suggested as an alternative, but the molecular mechanism of how *S. enterica* develops resistance to phages is still unclear.

Purpose: Assess the changes in the expression of 16 *S. enterica* genes after different phage multiplicities of infection (MOI) against *S. enterica*.

Methods: Phage cocktail SE14, SF5, SF6 at MOIs 1, 10, 100, 1,000 and 10,000 plaque-forming units (PFU) per colony-forming units (CFU) of four *S. enterica* strains (*S.* Enteritidis, *S.* Newport, *S.* Muenchen and *S.* Typhimurium) were added to 96-well plates and stored at 22°C ± 1°C for day 0 – 3. The RNA from the samples were extracted on days 0, 1 and 3 and RT-qPCR was used to determine the expression of the 16 selected genes. Sample size was 144.

Results: The expression of the *S. enterica* genes was dependent on the strain and MOI. Genes for the membrane transport system (*yedE*) was downregulated by 1.8-fold for *S.* Typhimurium but was upregulated by 1.4-fold for *S.* Enteritidis. Expression of genes involved in the outer membrane of *S. enterica* were found to be differentially expressed, *lolB* was downregulated by 2.7-fold but *yjbE* was upregulated by 2.7-fold for *S.* Typhimurium. Virulence genes (*invA*, *sopB* and *spvC*) were downregulated by 2 – 4-fold across the strains for MOI 100 and 1000. In contrast, genes involved in stress response and restriction modification (*hsdS*, *mod* and *res*) were upregulated by 0.5 – 2.5-fold across the strains for all MOIs.

Significance: The understanding into the transcriptional response of *S. enterica* after the phage treatment gives a further insight into how *S. enterica* can develop phage resistance and ways to target *S. enterica* more effectively.

P3-210 Food Contamination Incidences by Foreign Materials (FMs) Reported in Japan, 2016–2019

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Introduction: In Japan, complaints on food contamination by foreign materials (FMs) are reported to the municipal health centers. Each health center has archived data for the complaints, however these are not shared with others nor summed up, due to differences in reporting formats between municipalities. Nationally aggregated data are necessary to guide food business operators (FBOs) in introducing control measures against food contamination by FMs.

Purpose: To analyze the situation and the risk factors for food contamination by FMs in Japan.

Poster

Methods: Reported complaints on food contamination by FMs were collected for a period from December 2016 to July 2019 from 150 (97.4%) out of widely selected 154 municipalities in Japan by a questionnaire. We compiled the data by FMs, food items, food production/handling procedures related to the contamination, occurrence of health damage, physical size of the contaminants, and hard object contaminants.

Results: There were 14,684 complaints reported on food contamination by FMs during the data collection period (approximately 2.7 years). Most frequently reported FMs were bugs followed by metals, hairs, and plastics. Prepared dishes, confectionery and processed farm products were the most reported food items. 36.6% of the contamination events were estimated to have occurred in food production/handling/cooking business sectors with 26.8% of them due to hard objects. Prepared dishes, confectionery and processed farm products were the most reported food items for hard object contamination occurring in the food business sectors, and the most reported hard objects were metals followed by plastics and animal derived materials. 82% of the health damage events related to the contamination occurring in the food business sectors were caused by the hard objects.

Significance: These data enable us to recognize the situation of food contamination by FMs in Japan, and to guide FBOs in preventing food contamination by FMs.

P3-211 Inhibitory Effect of Clove (*Syzygium aromaticum*) and Green Tea (*Melaleuca alternifolia*) Essential Oils by Vapor Phase Against *Aspergillus flavus* in Corn

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Introduction: Mycotoxigenic fungi and their metabolites contaminate a wide variety of agricultural crops, from field to storage and distribution. Corn is susceptible to contamination by these fungi, specifically *Aspergillus flavus* and its metabolites, aflatoxins.

Purpose: To evaluate the inhibitory effect of clove (*Syzygium aromaticum*) and green tea (*Melaleuca alternifolia*) essential oils in vapor phase against *Aspergillus flavus* in corn.

Methods: Dry corn was inoculated with spores of *A. flavus* (3 to 4 log(CFU/g) aproximately). For the vapor contact assays, 50 g of inoculated corn were placed on petri dishes. On the plate cover a filter paper containing the different essential oils (125, 250 or 500 µL of EO/L of air) were placed. Dishes were incubated at 25ºC. A growth control was prepared in parallel to ensure that viable organisms were present. Corn grains were measured CFU/g in PDA every 5 days during 25 days. Minimal inhibitory concentration (MIC) was defined as the lower concentration tested were no fungal growth was detected. Every test was performed by triplicate. Gompertz modified model was applied in order to describe the inhibition.

Results: MIC values for both essential oils were at 250 µL of EO/L of air. The modified Gompertz model adequately described mold inhibition curves (mean coefficient of determination 0.941±0.03). Modeling show that for clove EO the main inhibition effect is in the first 5 days and it maintians no growth of A. flavus in corn. Green tea EO inhibits in 15 days and have the same fungicidal effect. Non MIC concetrations (125 µL of EO/L of air) for both EO, however an aproximately 1 log (CFU/g) reduction were detected.

Significance: Clove and green tea essential oils could inhibit *A. flavus* growth in corn by the action of the volatile compounds lowering the risk of developing mixotoxins.

P3-212 Application of Winter Savory Oil Emulsion to Control *Escherichia coli* O157:H7 in Inoculated Romaine Lettuce

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Introduction: Foodborne outbreaks due to *Escherichia coli* O157:H7 on lettuces have been increased over the years in the U.S. Natural antimicrobial such as essential oils (EOs) have been studied due to their strong antibacterial properties. Thus, winter savory EO emulsion may be an alternative to chemical sanitizers against foodborne pathogens.

Purpose: The aim of this study was to evaluate the antimicrobial efficacy of winter savory EO emulsion (WS) compared to chlorine and peroxyacetic acid (PAA) in a washing system with *E. coli* O157:H7 in inoculated romaine lettuces.

Methods: EO emulsions were prepared with sonication using Tween 80 as emulsifier. *E. coli* O157:H7 was inoculated on lettuces and then washed in a simulated postharvest system with 200 ppm of free chlorine, 80 ppm PAA, and 0.94 and 1.88 µL/mL of winter savory EO emulsion (WS1 and WS2, respectively), based on minimal inhibitory concentration results. Bacteria reduction on inoculated lettuce and cross-contamination after each treatment were also assessed during 0h, 24h, and 7 days of storage.

Results: Chlorine, PAA, and WS1 significantly ($p<0.05$) reduced *E. coli* O157:H7 population on inoculated lettuces over time (0h to 7 days), reaching 2.46, 2.07, and 1.68 log CFU/g reductions, respectively, compared to control (water) at 7 days. PAA was the most effective treatment in limiting *E. coli* O157:H7 cross-contamination at all three storage times, followed by WS2, chlorine, and WS1. The WS2 had a significant consistent reduction over time reaching levels below the limit of detection (-1 log CFU/g) at 7 days.

Significance: Winter savory oil may be a suitable alternative to the use of chemical sanitizers during the washing of romaine lettuces to reduce the *E. coli* O157:H7 on leaves and prevent cross-contamination.

P3-213 GIANT LEAPS Towards Healthy, Safe and Sustainable Future Diets by Filling Knowledge Gaps on Alternative Proteins – Policy Briefs

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Introduction: The major impacts of the current food system on biodiversity, land and water use, and animal welfare, could be mitigated by a shift from traditional animal-based towards more sustainable protein sources.

Purpose: The ambition of the GIANT-LEAPS project is to develop improved, fast, and animal-friendly methodologies for the health, safety and environmental assessment of alternative proteins to arrive at future diets that are accepted by consumers and optimized for health and environmental impacts within the scope of the project.

Methods: The research will focus on nine alternative proteins which are not commonly applied in foods (lentils, faba beans, oat, quinoa, rapeseed, microalgae, single cell bacteria, insects, cultured meat) to fill knowledge gaps as well as on a selected set of novel alternative proteins for specific safety-related aspects. This allows identifying the highest potential alternatives for specific cultures and target groups in the different regions of Europe.

Results: All missing elements of an integrated framework to evaluate protein sources are developed to enable the shift towards a sustainable food system and healthy nutrition: (i) innovative prototypes that unlock the potential of sustainable protein sources in foods, products and diets, (ii) predictive in silico and in vitro methodologies for safety and health parameters, (iii) datasets and cloud platform for data integration, analysis and comparison, and (iv) models to estimate and optimize the environmental and health impacts of future diets and the shift towards alternative protein foods. Policy briefs are one set of deliverables.

Significance: Around 60-70% of total protein intake is derived from animal sources in European diets. The ambition of the GIANT-LEAPS consortium is to achieve 50% of total protein intake derived from alternative protein sources, representing a decrease in absolute animal protein intake of 20-30% under the assumption that increased alternative protein intake fully substitutes traditional animal protein intake.

Poster

P3-214 A Fit-For-Purpose Evaluation of the GENE-UP® *Salmonella* (SLM) Assay in a Variety of Plant-Based Raw Ingredients

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Introduction: As consumer demand for plant-based food options grows, the food and beverage industry continues to search for the best blend of plant-based raw ingredients to provide their demanding customers with the best flavor and mouth-feel. This warrants the need for rapid and improved screening of pathogens such as *Salmonella.*

Purpose: In a series of validation and verification studies, we demonstrate the efficacy of the GENE-UP SLM2, a real-time PCR assay in screening 375g samples of several plant-based raw ingredients for *Salmonella* spp.

Methods: AOAC validation style studies were performed for two plant-based raw ingredients. Validation studies included 20 low (~0.0-2.0cfu) and 5 high (~5.0cfu) *Salmonella* spiked samples. Verification studies were performed on 6 additional plant-based raw ingredients and consisted of 7 replicates of each product spiked at ~3-20 CFU. All samples were enriched at 1:10 at 36℃ and 42℃ for 22-hours. For both study designs, samples were run on the candidate assay alongside the BACTVIAB™ PMAxx™, a residual DNA removal protocol. Samples incubated at 36℃ were compared to the FDA-BAM-Ch 5 and ISO-6579-1 (2017E) reference methods. The 42℃ incubated samples were compared to the FDA-BAM-Ch 5 method.

Results: Probability of Detection analysis at P<0.05 demonstrated no statistically significant differences for candidate vs. reference method positives in both validation studies and for candidate presumptive vs. confirmed results for verification studies. All studies demonstrated 100% alignment with culture confirmation regardless of screening method (candidate vs. traditional methods). For both study designs, samples screened with the candidate method were identical to the residual DNA removal protocol for both 36℃ and 42℃ enrichments.

Significance: Data presented here supports the claim that the GENE-UP SLM2 assay and BACTVIAB-PMAxx protocol offers a rapid and reliable routine screening of *Salmonella* in large samples of plant-based raw ingredients, enriched at either 36℃ or 42℃.

P3-215 Evaluation of a Rapid Alternative ATP-Bioluminescence-Based Method and Comparison with Traditional Methods to Detect Microbial Contamination in Plant-Based UHT Beverages in Argentina

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Introduction: Consumption of plant-based food has increased recently. Ultra-High Temperature (UHT) processes can be considered a good tool for extended shelf life. Commercial sterility should be tested to detect any failure during manufacturing process. ATP-Bioluminescence detection is a rapid alternative method to evaluate commercial sterility compared to traditional methods, like agar growth and pH. Argentinian regulations require pH measurement after incubation at 35°C for UHT product release.

Purpose: To evaluate an ATP-bioluminescence-based method (3M™ Microbial Luminescence System MLSII) and compare with pH and agar growth to detect microbial contamination on Plant-based UHT beverages.

Methods: Two matrices were evaluated (Coconut and Peanut) and four microorganisms were individually spiked (*P. aeruginosa, B. cereus, L. plantarum, S. cerevisiae*). Overnight cultures were inoculated at two levels: 0-10 and 10-50 CFU/container. All samples were incubated at 37°C for 48 and 96h. Seven samples were used for each matrix/microorganism/incubation time. Additionally, 30 samples were used to determine background ATP and pH. After incubation, samples were tested with MLSII, pH and agar growth ((MRS agar for *L. plantarum*, YEPD agar for *S. cerevisiae*, TSA for *B. cereus* and *P. aeruginosa*). Differences among methods were evaluated using chi-square test.

Results: 92 out 148 spiked samples yielded positive by both MLSII and agar. All the samples reported positive by agar were confirmed as positive by the MLSII, no false positives were found. For pH measurement results were statistically different than agar and MLSII (X^2>3.84). Twenty-two positive samples did not have enough pH change to considered as positive. All the samples spiked with *S. cerevisiae* did not have growth detectable by any method. All the negative results were reported as negative.

Significance: The MLSII ATP-bioluminescence-based method is a reliable rapid alternative method for Plant-based UHT beverages release in Argentina, with equivalent or better results than agar or pH.

P3-216 Rapid Detection of *Cronobacter* Species in Non-Dairy Plant-Based Products Using the ATP Detection Innovate System

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Poster

Introduction: This study demonstrates detection of *Cronobacter sakazakii* and *Cronobacter muytjensii* in 6 different plant-based dairy alternatives.

Purpose: Ensure that new pathogens are detectable using ATP methods in non-dairy plant-based products.

Methods: Bacteria were grown and diluted into 6 products: ESL Milk - Chocolate, Low fat and Original, UHT Milk – Chocolate and Original, and High fat milk where each milk had differing ingredients and fat content and represented a range of flavors and colors. Bacteria were added at 2 levels, a high CFU (approximately 10,000 CFU per pack or 10 CFU per 1mL)- and a low CFU (<100 per pack or < 1 CFU per 10 mL). Each pack was incubated at 35°C, aliquots removed and examined for ATP for 3 consecutive days using RapiScreen™ Dairy and confirmation plated onto TSA.

Results: Each milk type produced rapid growth in the packs at 24 hours, the comparison of growth rate using RLUs shows the following: ESL and UHT Original had mean RLUs of 99,880 at high spike and 74,660 for low spike and 114,928 and 128,786 RLUs at 24 hours, respectively. At low spike, ESL and UHT Chocolate had 134 RLUs and 174 RLUs at 24 hours, rising to 90,537 RLUs and 29,407 RLUs, respectively, at 48 hours - the chocolate milk appears to slow down the growth and subsequent ATP titer. For ESL-Low fat, and UHT-High fat milks, the low spike was 7,804 RLUs and 33,316 RLUs, respectively. Both species tested were easily detected in all 6 products, producing 100s of RLU above the baseline, showing detection within 24 hours.

Significance: Cronobacter spp. can be rapidly detected in plant-based milk using the Innovate System ATP rapid method.

P3-217 Hygiene Management Level Applied in Meat Areas by Supermarkets in Mexico

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✦ ***Undergraduate Student Award Entrant***

Introduction: The level of hygiene applied in perishable food handling at retail is an important issue; furthermore, that cross-contamination could occur and constitute a consumption risk.

Purpose: Assess the level of hygiene in the meat product areas of different supermarkets.

Methods: A visual hygienic-sanitary evaluation was carried out every Sunday from January to May 2022 in four supermarkets from Texcoco de Mora, State of Mexico, focused on meat product areas. It consists of a five-point scale (0=very bad to 5=very good). Data were analyzed under a completely randomized design using categorical variables. Grouping means between categories was done at a probability of p<0.05 using the chi-square test.

Results: Hygiene management problems were detected in some evaluated areas of supermarkets. The use of appropriate uniforms by employees was found to be poor with 17%, while temperature management in the fresh meat cold stores was found to be very poor and poor with 7.95 and 28.41%, respectively.

Significance: Currently, there is a risk of cross-contamination in all supermarkets evaluated. Training is required for the meat area staff to increase the level of hygiene.

P3-218 Updated Assessment of State Food Safety Laws for Norovirus Outbreak Prevention in the United States

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Introduction: Foodborne norovirus outbreaks are frequently associated with contamination of food during preparation by an ill employee, often through barehand contact with ready-to-eat (RTE) food. The US Food and Drug Administration's Food Code outlines model food safety provisions to prevent illness transmission in retail and foodservice establishments. The Food Code is updated every 4 years; adoption of specific provisions is at the discretion of state governments.

Purpose: We updated, as of March 2020, our 2014 assessment of state adoption of key norovirus-related food safety provisions in the United States.

Methods: Food safety laws of the 50 states and District of Columbia were assessed for adoption, as of March 2020, of four norovirus-related provisions included in the 2017 Food Code: 1) prohibition of barehand contact with RTE food, 2) exclusion of food employees with vomiting or diarrhea, 3) person in charge (PIC) as a certified food protection manager (CFPM), and 4) written response plan for vomiting or diarrheal contamination events.

Results: The provision prohibiting barehand contact with RTE food was adopted by 45 states (88%), an increase from 39 states (76%) in 2014. Forty states (78%) require exclusion of food employees with vomiting or diarrhea for ≥24 hours after symptom cessation, an increase from 30 states (59%). The requirements for a PIC as a CFPM and written response plan for contamination events were newly added to the 2017 Food Code. Five states (10%) require a PIC as a CFPM, and nine states (18%) require a written response plan for contamination events.

Significance: Adoption of provisions prohibiting barehand contact with RTE food and exclusion of ill food employees increased. Newer provisions requiring a person in charge as a CFPM and a written response plan to contamination events were not as widely adopted. Increased adoption of updated Food Code provisions and improved compliance may decrease foodborne norovirus transmission.

P3-219 Characterization of Foodborne Pathogens Isolated from Select Meat Products and Ethnic Food Products Marketed in Food Desert Areas of Central Virginia

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Introduction: In food desert areas, low-income households without convenient transportation often shop at small, independently owned corner markets and convenience stores (SIOM). Studies indicate a high potential for reduced product quality and safety of foods sold at SIOM, with more critical and non-critical code violations in the region.

Purpose: This study assessed the difference in market type on the microbiological quality in select food products procured from food deserts in Central Virginia.

Methods: A total of 326 samples procured from registered 10 SIOM and nine large chain supermarkets (LCSM) between August 2018 and March 2020 were evaluated for the prevalence of and antimicrobial resistance of foodborne pathogens.

Results: Higher levels of aerobic mesophile and coliform counts were found in SIOM-acquired samples than in LCSM-acquired samples, as demonstrated by the lower food safety compliance rate of SIOM. Regardless of SIOM or LCSM, *Campylobacter*, *E. coli*, *Listeria*, and *Salmonella* were detected in samples of 3.6%, 20.9%, 5.5%, and 2.7%, respectively. Most *Campylobacter* (75%, 6/8) and *Salmonella* (83.3%, 5/6) detected were from SIOM-acquired samples, including ethnic food products. Among the tested antimicrobials, AMP (100%) and TOB (100%) showed the highest frequency of resistance among *Campylobacter*, TCY (69.9%) among *E. coli*, NAL (100%) among *Listeria*, and TCY (50%) among *Salmonella*, respectively. The prevalence of multi-drug resistance (MDR) and non-susceptibility in *Campylobacter* and non-susceptibility in *Listeria* isolated from SIOM-acquired food products were lower than those isolated from LCSM-acquired samples.

Significance: The higher price of the same brand name commodity sold at SIOM than those sold at LCSM was observed manifesting an increased burden to economically challenged residents in food desert areas. Elaborated and in-depth research on a larger-scale sample size with a greater diversity of products is needed to determine and intervene in the cause(s) of the observed differences in the prevalence of the pathogens and AMR profiles.

P3-220 Prevalence of *Listeria monocytogenes* on Food Contact and Non-Food Contact Surfaces in Fresh Food Markets of Asuncion, Paraguay

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❖ ***Developing Scientist Entrant***

Introduction: Foodborne illnesses represent a significant burden for developing countries, however lack of available data makes it difficult to address. In Paraguay, between 1993-2010, only 45 outbreaks were reported. To date, no outbreaks have been linked to *Listeria monocytogenes*. Nevertheless, *L. monocytogenes* represents a threat to public health. To understand the risk of *L. monocytogenes* contamination in the Paraguay vegetable chain, it is important to evaluate pathogen prevalence on food contact and non-food contact surfaces.

Purpose: This study aims to determine the prevalence of *L. monocytogenes* on food contact and non-food contact surfaces in fresh food markets in Asuncion, Paraguay.

Methods: A total of 92 vendors were sampled in multiple fresh food markets during winter (June) and summer (November-December) seasons of 2022. For each vendor, 2 food contact surfaces and 2 non-food contact surfaces were swabbed. Samples were screened for *L. monocytogenes* presence utilizing Buffered *Listeria* Enrichment Broth, and selective agars (CHROMagar *Listeria* and Modified Oxford Agar). Typical colonies were streaked on non-selective agar and stored for further analysis. Presumptive positive isolates were confirmed using PCR and results recorded for further analysis.

Results: A total of 14 vendors (15.2%) had at least one surface type test positive, and 3 vendors (3.2%) had both surface types test positive for *L. monocytogenes,* demonstrating the risk that this pathogen poses to public health. Overall, the prevalence of *L. monocytogenes* for each food contact surface and non-food contact surface was 4.9% (9/184). Additionally, most positive results (15/18) were collected during summer, which can be attributed to higher temperatures that provides a better environment for the pathogen to thrive.

Significance: This study is a first step to understand prevalence of *L. monocytogenes* in fresh food markets in Paraguay. Utilizing this data, regulatory bodies can develop surveillance systems to improve food safety and implement strategies to prevent foodborne illnesses.

P3-221 A Survey and Microbiological Tests for Development of Meal Kits Using in Children's Foodservices

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Introduction: Recently, meal kit was newly established as a new food type in Korea.

Poster

Purpose: In order to develop a meal kit for children's foodservices, a survey was conducted on the necessity and intention to use the meal kit for managers of children's foodservices in Changwon city. In addition, three recipes with high frequency of serving at children's foodservices were prepared in meal kit packaging and refrigerated below 5°C, and microbial tests were conducted on the same day (0 day), 3rd and 5th day to review safety.

Methods: A total of 141 people (50.3% response rate) responded to the e-mail survey conducted from December 2020 to April 2021. A meal kit for five children serving was produced with three recipes for children aged 5～7 years old (pork bulgogi, seasoned spinach, radish salad). Quantitative analysis (Aerobic bacteria, Coliforms, *E. coli*, *Staphylococcus aureus*) was performed according to the Korean Food Code, and *Salmonella* spp, *L. monocytogenes*, Shiga toxin producing *E. coli*(STEC) was performed by 3M™ Molecular Detection System.

Results: The opinion that "meal kits are necessary for the operation of foodservices" was significantly higher in small sized foodservice managers with less than 50 meal counts (69.9%) than in the foodservice managers with more than 50 meal counts (52.9%) ($P<0.05$). Nevertheless, 62.1% of those surveyed said they would not use meal kits for children's foodservice at present due to safety concerns. On day 3 of refrigeration, Coliform counts were significantly increased in the pork meal kit ($P<0.001$), in the seasoned spinach: Aerobic bacteria counts (P<0.001), Coliform counts ($P<0.001$) and *Staphylococcus aureus* ($P<0.001$), in the radish salad: Staphylococcus aureus ($P<0.001$).

Significance: In the future, strict hygiene standards for meal kits for children's foodservices should be established, and the HACCP system, including sanitation treatment, must be applied to reduce microbial levels.

P3-222 The Influence of Customer Focus on Food Safety Behaviour in Food-Service Sector.

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Introduction: One of the main goals of the food-service sector is to satisfy customer demands alongside taking all reasonable steps to ensure food safety. Gaining understanding about the influence of customer focus on food-handler food safety behaviour is important for establishing an effective food service environment and positive food safety culture.

Purpose: The study aimed to explore how customer focus may impact food-handler food safety compliance using qualitative analysis of stakeholder perceptions.

Methods: Three groups of stakeholder interviews (experts *n*=11; management *n*=9; employees *n*=4) were qualitatively analysed. Perceptions, such as business targets, customer-oriented goals, customer expectations, customer pressure, risk perception and improvement suggestions were compared among the groups with considerations for food safety implementation.

Results: This study determined different prioritisation among the groups, whereby the management target long term profits and reputational gains, but employees focus on immediate day-to-day tasks. Experts indicated the main goal is that "*the customers receive safe food*", whilst management focused on "*experience over just a transaction*". In turn, customer expectations were said to grow and create goals which are unrealistic for the employees to meet in a given time and may make them prioritise service over food safety practices: "*If they're impatient -we end up getting hastened and skip the task*". Overall, stakeholders indicated the need to simplify the tasks and communicate to the customers that food safety requires time. However, redirecting focus from customer demands may be challenging.

Significance: This study determined the need to raise customer awareness that time is vitally important for food-handlers to carry out their tasks in ensuring food safety in food-service establishments. Tasks must be simplified, depending on the nature of the business, to be achievable, whilst prioritising food safety implementation. This way customer focus could be balanced with food safety compliance and could drive food safety culture and safeguarding customers' health.

P3-223 Perceived Benefits and Limitations of Proposed AI Food Safety Monitoring Software in Food Service Sector.

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Introduction: Ensuring the safety of food is paramount for food service establishments and novel Artificial Intelligence (AI) software utilising CCTV footage could provide real-time feedback and revolutionise food safety monitoring in food service. However, the perceptions of the stakeholders about the use of such software in food service must be explored, as previous research mainly focused on food manufacturing sector.

Purpose: To determine stakeholder perceptions regarding the benefits and limitations of novel AI food safety monitoring; and to identify the requirements of the intended users regarding the development of such technology.

Methods: In-depth qualitative interviews were conducted (*n*=24) with stakeholders: experts, management and employees working within food service. Qualitative thematic analysis was used to determine stakeholder benefits/limitations perceptions and suggestions for technology introduction to the business.

Results: Stakeholders expressed positive sentiments and interest in the development of AI food safety monitoring technology: "*I think real-time feedback is beneficial*". Benefits, such as: control over monitoring, continuous real-time feedback, speed and transparency, visible feedback and others were highlighted. Limitations to using novel technologies such as: time and financial investment, need for maintenance and review, ethical concerns and others were discussed by the stakeholders. Suggestions for development and introduction of novel software to food service business were made: "*be very clear about what you're trying to do and what you're not trying to do*".

Significance: This study has identified perceptions of stakeholders about the use of AI monitoring software in food service, and practical considerations when developing such novel technology and introducing it to the business. Importantly, benefits are seen when technology is used supplementarily and when its purpose is clearly communicated to the personnel. These findings could inform the future development of AI food safety monitoring technology to improve food safety in food service sector.

P3-224 The Influence of Previous Experience on the Culture of Food Safety in Food-Service Establishments in England and Wales

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Introduction: Expected food safety behaviour is defined and demonstrated by the managers in food-service establishments. Considering the transient nature of the industry, it is important to explore the impact of previous experience of managers on the food safety behaviour modelled by them.

Purpose: To explore how previous experience, such as training; length of employment and seniority; personal experience of food safety incidents may influence the way food safety culture is established in a business with considerations for behavioural change.

Methods: Food-service management interview data (*n*=21) from England and Wales were analysed using thematic analysis according to Food Safety Culture indicators. The Health Belief Model was used to qualitatively explore and structure how previous experience may impact future actions of influencing a positive food safety culture.

Results: Lack of awareness about food safety culture as a concept was indicated by the managers. Length of work experience was reported to result in habits: "*lots of people got so set in their ways*" leading to behaviour norms passed on to the junior staff "*you end up communicating 'this is what we do'*".

Poster

Increased risk awareness was reported by managers with previous exposure to foodborne incidents: "*I want to make sure that no one else has the same experience as I did*". Previous experience was also reported to impact participants' motivation, perception of susceptibility, and aspects of food safety culture, such as communication and leadership.

Significance: This qualitative study has demonstrated that, depending on its nature, previous experience may be a barrier to a positive food safety culture; or, alternatively, as a cue to action, it may encourage and motivate food safety culture improvement in food-service setting. Food safety culture should become an integral component of management training, designed to highlight the benefits of establishing a positive food safety culture in a business and complementing/counteracting previous experience.

P3-225 An Examination of Food Handling Practices at Food Pantries Across Virginia and Suggestions for Improving Food Safety Practices in the Food Pantry Setting

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❖ ***Developing Scientist Entrant***

Introduction: This study will collect observations of food pantries in Virginia in accordance with various food safety guidelines in order to provide suggestions about how food pantries can operate more safely..

Purpose: The objectives of this study were to identify the most common risky food handling practices observed at food pantries, and to use this information to develop clear educational tools that can be used to educate pantry managers and volunteers how to ensure that the food provided to their clients is safe and wholesome.

Methods: Twenty pantries across the Virginia were observed during operation to record food handling practices. Data was collected using a rubric which was developed by combining resources from Virginia Department of Health, National Restaurant Association, and FDA Model Food Code. In addition to observation data, each pantry's manager completed a survey to measure basic food safety knowledge as well as further describe operational practices.

Results: The three riskiest food handling practices included 1) perishable food holding time, 2) personal hygiene of volunteers, and 3) using correct handwashing practices.There were only two handwashing attempts observed between all 20 pantries and neither of these attempts was correct. 8 of 20 (40%) of the pantries did not keep up to date records of their refrigerator and freezer temperatures. 9 of 20 (45%) of pantries had someone on site using a personal item while handling food, and 4 of 6 (67%) of pantries that prepared or cooked food on site had volunteers improperly wearing gloves during these activities. A summary sheet and checklist was developed that could help pantry managers and staff pay more attention to food safety at their organizations.

Significance: The results of this study help to identify the gaps of food safety knowledge in food pantry managers and volunteers and provide suggestions for how the Virginia Cooperative Extension can help to increase food safety knowledge and practice among pantry staff across Virginia.

P3-226 Challenges and Priorities When Serving Customers with Food Allergies in Private Clubs

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Introduction: Food allergies have become a serious public health concern in the U.S. with the increasing number of individuals with food allergies. The wide variety of food allergens and the potentially life-threatening effects of food allergy reactions have posed challenges for foodservice operators when accommodating these customers. Private clubs are a unique and special segment of the hospitality industry, and serving customers with food allergies and satisfying the special dietary needs of customers was identified as one of the challenges.

Purpose: This study aimed to explore the current issues and challenges related to serving customers with food allergies in club foodservice operations.

Methods: To achieve variation within the sample, a purposive sampling method was employed to recruit club foodservice managers from different states in the U.S. Interview questions were developed based on the literature review following the qualitative interview guidelines. The thematic analysis method was employed to identify, review, and refine the themes of transcribed interview data.

Results: A total of 15 club managers, directors, or executive chefs participated in the individual phone interviews. The average length of interviews was 21 minutes. Results indicated that food allergies posed challenges for club managers regarding the increasing populations and types of food allergies, the difficulty of preventing cross-contacts in small areas, and limited food allergen labeling on food products. Participants reported various strategies for preventing food allergy reactions, and the member information management system was most frequently mentioned (N=15). Other strategies include reassuring about allergen-free orders, involving managers in the communication line, and labeling key allergens on the menu. Balancing safety and satisfaction is critically important due to the nature of club operations.

Significance: This study provides important and valuable implications for club operators regarding how to cope with challenges when preparing safe meals for customers with food allergies.

P3-227 Motivating Foodservice Employees to Learn about Food Allergies: A Food Allergy Story Video Can Make a Difference

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Introduction: Food allergy, an immunological response to food proteins that may be harmful to the human body, is affecting more than 15 million individuals in the U.S. Dining out may be challenging for these individuals as studies found that foodservice employees lack knowledge about food allergies and ways to prevent cross-contacts.

Purpose: This study aimed to examine the effectiveness of a food allergy story video (featuring parents of a food allergy victim) on foodservice employees' motivation to learn about food allergies and training transfer intentions.

Methods: A self-administered online survey instrument was developed based on previous literature and was sent through Qualtrics to their survey panel of foodservice employees in the U.S. SPSS was used for data analyses. The food allergy story video was edited from Caroline's Story video developed by Kwon and Sauer (2015). MANOVA was performed to compare the dependent variables between the two groups (with vs. without video). PROCESS v4.0 by Andrew F. Hayes was used to test the mediating effects.

Results: A total of 410 valid survey responses were collected, with 204 participants assigned to watch the food allergy story video and 206 participants without the video. Results of the MANOVA test indicated that the food allergy story video has a significant main effect with a Pillai's Trace value of 0.07, $F=16.08$, $p<0.001$. Foodservice employees who watched the video had significantly higher motivations to learn about food allergies ($M=4.46$, $SD=0.79$) and higher training transfer intentions ($M=4.66$, $SD=0.60$) than those who did not watch a food allergy story video ($M=4.13$, $SD=0.83$; $M=4.24$, $SD=0.89$). For foodservice employees who watched the video, their cognitive empathy mediated the relationship between their motivations to learn and training transfer intentions ($p<0.001$).

Significance: This study's results provided useful implications for restaurateurs to increase employees' motivation to learn about food allergies and their training transfer intentions.

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P3-228 How Do Certified Food Protection Managers Impact Inspection Performance? a Retrospective Analysis of Inspection Records from Franklin County, Ohio

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❖ ***Developing Scientist Entrant***

Introduction: Previous analyses have shown that Certified Food Protection Managers (CFPM) are generally associated with overall retail food safety inspection performance; however, analyses often do not account for the variation in enforcement that is likely to occur between when inspections are conducted by different Registered Environmental Health Specialists (REHS).

Purpose: The objective of this study is to identify inspection items that are associated with a CFPM violation, while controlling for any differences in enforcement by REHS.

Methods: Records for all 2018 and 2019 standard inspections conducted at risk level III and IV food establishments licensed by Franklin County Public Health (FCPH) were obtained and totaled according to criticality, section of the Ohio Uniform Food Safety Code, and inspection item (e.g. Proper date marking and disposition) from the State of Ohio Food Inspection Report. Logistic regression was used to model the likelihood of a violation of each section or item based on the presence of a CFPM violation while controlling for differences in inspector.

Results: Approximately 3,774 inspections were conducted by nine FCPH-REHS during the study period. Failure to comply with the CFPM requirement was associated with an increased likelihood of many violation sections and individual inspection items. Inspections with a CFPM violation were 1.56 [95% CI:1.21-2.008] times more likely to have a violation of proper date marking and disposition, and 5.23 [95% CI: 4.10-7.180] times less likely to have a violation for procedures for responding to vomiting and diarrheal events.

Significance: By understanding how the current CFPM certification impacts establishment compliance while controlling for individual variations in enforcement, areas of poor compliance can be identified for increased emphasis in the CFPM training course or more frequent re-certification.

P3-229 Food Handlers' Beliefs about Food Safety Behaviours

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Introduction: Food handlers play an essential role in food safety, so it is important to understand the modal beliefs that drive their behaviours.

Purpose: To investigate food handlers' beliefs regarding safe food handling behaviours.

Methods: A qualitative study was carried out by interviewing 25 food handlers from a Brazilian Air Force's food service. To identify behavioural beliefs, participants were asked to list the advantages and disadvantages of safe food handling. To identify normative beliefs, participants were asked to list the groups of people who approve or disapprove of safe food handling. To identify control beliefs, participants were asked to list factors or circumstances that might hinder or facilitate safe food handling. Interviews were recorded, transcribed, and analysed using thematic content analysis, which resulted in qualitative categories. The Brazilian Ethics Committee approved the project under the number 0276/2022.

Results: The mean age of participants was 37 years (Standard deviation: 15), and 24 (96%) were men. The categories regarding behavioural beliefs that emerged from the analysis were "Perception of food quality and safety", "Time expenditure", and "Consumer health". The categories regarding normative beliefs were "Management and nutritionist", "Food service employees", "Sector managers", "Auditors". The categories regarding control beliefs were "Proper equipment and utensils", "Work overload", "Sanitization of equipment and utensils", "Personal hygiene", and "Adequate work space". The food handlers' behavioural beliefs were related to the external locus of control, the normative beliefs were related to the people involved in the process, and the control beliefs were determined by resource management and work pressure. Food handlers' beliefs about safe food handling behaviours were characterized and stated by the work of other employees and the environment.

Significance: Twelve food handlers' beliefs regarding safe food handling behaviours were identified based on qualitative categories. These beliefs may be useful in developing food safety educational actions.

P3-230 Food Safety Practices Among Ohio Establishments Utilizing Food Delivery Systems

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Introduction: Retail food delivery systems have gained popularity within recent years, and while direct-to-consumer (DTC) systems are regulated by local health authorities, food safety regulations surrounding third-party delivery (TPD) services (e.g., DoorDash, Grubhub) are inconsistent in Ohio.

Purpose: The aim of this study is to identify food safety practices within food delivery systems in Ohio food service establishments utilizing food delivery systems.

Methods: Owners and managers of retail food service establishments who are members of the Ohio Restaurant Association (ORA) listserv were recruited in January 2022 to participate in an anonymous, online Qualtrics survey about their food delivery systems and related food-safety practices (e.g., packaging methods, time/temperature control, driver training requirements). A total of 76 ORA-affiliated owners and managers responded to the survey. Associations between establishment characteristics and food delivery practices were examined using logistic regression.

Results: Of the 76 respondents, 55.26% offered TPD services only; 35.53% offered DTC systems only; and 9.21% offered both. Establishments using TPD systems were less likely to utilize insulated delivery bags (OR=0.1563; 95% CI=(0.0536, 0.4554)) and coolants (OR=0.0697; 95% CI=(0.008, 0.6057)) than establishments offering DTC systems. Establishments offering catering were significantly more likely to use insulated delivery bags (OR=6.4; 95% CI=(1.0165, 40.294)) and coolants (OR=13; 95% CI=(1.7861, 94.621)) in their delivery systems than establishments that offer full-service dining.

Significance: Research on food safety knowledge and practices within food delivery systems is limited nationally. Understanding retail food and food service establishment practices and logistics of regulating TPD systems will allow education and regulatory efforts to be focused on clarifying feasible food safety practices within food delivery systems.

P3-231 Trending FDA Inspectional Observations from FY2006 to FY2022: *How Can This Data Help Food Facilities Prioritize and Focus on Key Food Sanitation Control Programs?*

Amit Kheradia

Remco: a Vikan company, Zionsville, IN

Introduction: FDA's Office of Regulatory Affairs have been using the inspection-based Form 483s to cite violations observed within FDA-regulated production facilities. Interestingly, over 50% of the 483s published are related to food products, including dietary supplements.

Purpose: To trend 17 years of pooled FDA 483s on food facility inspectional data, and to assess this data with regards to the key citations related to equipment and environmental sanitation.

Methods: Published data of FDA 483 Observations from FY 2006 to FY 2022 was used and, from each year, the top 50 citations were extracted. The extracted citations were then further refined to select those relevant to environmental and equipment sanitation, and the frequency of each of these sanitation violations was noted. Owing to the phased implementation of FSMA rules, transition from 21 CFR110 to 21 CFR117 was observed starting FY 2016. Full transition was in effect from FY 2018 onwards.

Results:

- On average, about a third of the observational violations and frequencies in the top 50 citations were relevant to the study, as defined above.
- The recent citations with the highest frequencies were: 21 CFR117.35(c), 21 CFR117.80(c), and 21 CFR117.35(a).
- 12 key environmental and equipment sanitation issues were identified following data analysis, the top three being: (1) pest control, (2) inadequate precautions to control cross-contamination during processing operations, and (3) failure to maintain the plant under sanitary conditions.

Significance: This study shows the importance of inspectional visits and reporting by highlighting the key sanitation issues identified in the food industry. This reliable and readily available information from the FDA can be used proactively by industry to prioritize their resources on high-impact sanitation control measures based on risk. In turn, this should help the industry improve their production environment, equipment, processes, and product safety programs, and significantly protect them against potential product recalls or loss of business.

P3-232 Efficacy of Commercially Available Sanitizers to Control *Salmonella* Biofilms on Harvesting Bins and Picking Bags

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❖ ***Developing Scientist Entrant***

Introduction: The ability of *Salmonella* to form biofilms is predicted to be a strategy to increase persistence and survival. When harvesting tools and bins are not effectively cleaned and sanitized, microorganisms can persist and potentially cross-contaminate produce.

Purpose: Evaluated the efficacy of five commercially available sanitizers to control *Salmonella* biofilms on experimentally inoculated coupons [nylon, high-density polyethylene (HDPE), and wood] representative of harvesting bins and picking bags.

Methods: Multi-strain *Salmonella* biofilms were grown in a Centers for Disease Control and Prevention reactor at 22± 2°C for 96 hours. Three *Salmonella* serovars were used: *S.* Enteritidis, *S.* Agona and *S.* Newport. Biofilms were exposed to 500 ppm free chlorine, 500 ppm peroxyacetic acid, 75psi steam, and 4% silver dihydrogen citrate (SDC) for 1 or 2 minutes and 400 ppmv chlorine dioxide for 24-hours. Treated samples were placed in a neutralizing solution, cells were detached by sonication and vortexing, serially diluted, spread plated, and incubated for 24 hours at 37 ± 2˚C. Treatments were replicated six times.

Results: Nylon, HDPE, and wood control coupons resulted in initial *Salmonella* populations of 9.55, 8.73, and 9.29 log CFU/coupon, respectively. All treatments significantly impacted viable *Salmonella* populations when compared to the controls ($P<0.05$). Viable cells were undetectable (<2.40 log CFU/coupon) for all chlorine dioxide treatments. Application time did not affect the efficacy of steam ($P>0.05$). The 1- and 2-minute application time resulted in a significant difference of viable cells for PAA on nylon (6.10 vs. 2.98 log CFU/coupon) and wood (6.42 vs. 4.88 log CFU/coupon), chlorine on nylon (7.44 vs. 6.54 log CFU/coupon) and HDPE (4.14 vs. 3.18 log CFU/coupon), and SDC on HDPE (5.96 vs. 4.82 log CFU/coupon) ($P<0.05$).

Significance: Understanding the efficacy of sanitizers to control *Salmonella* biofilms may improve the safety of tree fruit crops by increasing available treatment strategies to sanitize picking bags and harvesting bins.

P3-233 A Sanitation Validation Case Study Highlights Industry Challenges for a Small, Fresh-Cut Processor

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Introduction: A small value-added processor recalled a large order of refrigerated diced sweet potatoes due to spoilage contamination where multiple contributing factors were hypothesized in a root cause analysis.

Purpose: This case study highlights a sanitation validation study conducted on a hard-to-clean dicer, as well as the challenges of implementing adequate sanitation programs among small processors.

Methods: The validation study began with an evaluation of the dicing equipment design and review of written SSOPs, including in-person observations of employee practices. Environmental swab samples were also collected before sanitation and after sanitation (3 rounds) from 26 strategically-located food contact surfaces, based on difficulty of cleaning, equipment design, and product buildup (N=78). Samples were enumerated for total aerobic bacteria (APC), coliform, and yeast and mold using 3M™ Petrifilm™. Acceptable microbial criteria thresholds were based on industry standards.

Results: A review of written SSOPs revealed a lack of instructions on equipment disassembly with limited details regarding tools and specific sanitation procedures. In-person observations confirmed nonconformance with sanitation procedures as evidenced by high-risk activities (e.g., use of high-pressure hose) and inadequate cleaning of the dicing equipment. Round one (pre-sanitation) swab results (n=26) for microbial indicator groups identified 22 (85%) areas of excessive microbial buildup during production. Rounds two and three (post-sanitation) swab results (n=52) identified 8 (31%) and 11 (42%) sampled areas, respectively, above acceptable criteria. Although the third round samples reflected SSOP revisions, employee training, and further disassembly of equipment, results indicate a need for continued corrective measures and ongoing analysis.

Significance: This case study highlights sanitation and workforce barriers leading to sanitation nonconformances among many food processors. The need for education and technical services for small, rural processors is significant, as federal agencies such as the USDA Farm to School Program, continue to promote economic opportunities for producers and increase access to locally-sourced foods.

P3-234 Factors That Influence Staff Compliance with Cleaning and Disinfection Practices in a UK-Based Small and Medium Sized Enterprise (SME) Ready-to-Eat Food Manufacturer.

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Introduction: Cleaning practices in the food industry have been traditionally improved through technological advancements focusing on hygienic design. Focusing on factors that influence cleaning staff's compliance with cleaning practices may enable development of more cost-effective targeted interventions.

Purpose: Measure perceptions of food-handlers and management and factors influencing cleaning practices in a food manufacturing company to identify potential barriers and facilitating factors to support intervention development.

Methods: In-depth interview data(n=13) informed development of a quantitative survey to assess factors associated with cleaning within the company. The survey was distributed to all staff linked with cleaning practices in a(n=35 staff) SME ready-to-eat food manufacturer. Paper-based and digital-format surveys to increase accessibility were used and incentives were provided for completion to improve response-rate.

Results: All staff involved in cleaning activities (n=20) in the SME completed the survey. Overall, a positive sentiment towards food safety and importance of cleaning practices was indicated by the majority (90%) of staff. While staff reported awareness of relevant pathogens(100%) and allergens(95%), the perception of risk was low, with 50-62% managers/food-handlers perceiving a low likelihood of presence for *Listeria monocytogenes* and milk allergens. In addition, 75% strongly agreed that current cleaning practices can prevent microbiological and allergen contamination of product. Staff reported appli-

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cation of disinfectant and rinsing of detergent as the least important factors in ensuring adequate cleaning. The majority of staff indicated they do not use the documentation containing the cleaning instructions, with 73% reporting they learned cleaning procedures, hands-on, from a co-worker. Sixty-percent did not know the correct order for cleaning steps. Findings indicate the need to address the lack of knowledge and improve awareness of the importance of each cleaning step.

Significance:

Identifying factors that have a high likelihood of improving compliance with the cleaning practices by considering perceptions of both food-handlers and the management is essential for developing targeted interventions.

P3-235 Evaluating the Cleaning Performance of Various Surface Sanitizers Against Tough Kitchen Soils

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Introduction: Many food contact surface sanitizers have cleaning properties. However, an evaluation of the cleaning performance of these products testing has not been performed.

Purpose: To evaluate the cleaning performance of seven commercially available food contact surface sanitizers using standardized methods with kitchen soils.

Materials and Methods: Testing was performed according to Household and Commercial Products Association DCC17 (2018) "Greasy Soil" method. Briefly, a blend of lard, olive oil, carbon black, clay, flour, milk and water were baked onto porcelain tiles. Then, 15 grams of product was applied to soiled tiles and 10 cycles of wiping was performed using a sponge applied to a scrubbing machine standardizing wipe pressure and speed. Percent soil removed relative to non-cleaned tiles was calculated using reflectance measurements. Five replicates were conducted per product. Three quaternary ammonium compound-based, two alcohol-based, and two acid-based surface sanitizers were chosen. Water and a heavy duty degreaser were controls.

Results: The ethanol- and citric acid-based products removed 67.00 and 70.74% of all soil, respectively, which was significantly more than the other products ($p < 0.05$), but significantly less than that of the heavy duty degreaser (89.67% removal; $p < 0.05$). The remaining lactic acid, isopropanol, and three quaternary ammonium compound-based products removed 20.46 to 36.12% of all soil, which was statistically less than the ethanol- and citric acid-based products ($p < 0.05$).

Significance: The cleaning performance of test products varied greatly, with soil removal results likely tied to inactive ingredient formulation. Cleaning of food contact surfaces prior to application of a sanitizer is a critical step in the sanitation process. Given that many surface sanitizers are labeled such that they can also be used as cleaners, it is important to understand their relative performance to one another, especially since no current regulatory standard exists for cleaning performance.

P3-236 Use of Fluorescent Soil to Evaluate Cleaning Effectiveness of Food Temperature Probes

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Introduction: Insufficient cleaning of temperature probes between uses increases the potential for cross contamination in food preparation settings.

Purpose: The use of a fluorescent gel, as a surrogate food soil, was compared with a traditional food soil to evaluate probe cleaning effectiveness.

Methods: Two commonly used temperature probe designs and three commercially available probe cleaning wipes were evaluated in this study. Probes were contaminated by submerging the food contact portion of the probes into a 15 ml test tube containing either a food soil (ketchup) or a fluorescent gel (Glo Germ™). Following contamination, three probes were wiped consecutively with one wipe using a standardized procedure. The wipe was folded around the probe and wiped from top to bottom using a twisting motion for a total of two times, using a clean surface of the wipe for each pass. Following wiping, a visual assessment of cleanliness was performed using a four-point scale ranging from 0=no soil to 4=heavy soil. A black light was used to visualize remaining fluorescent contamination using the same four-point scale. Testing was performed under two test conditions, immediately following contamination and one hour post contamination.

Results: All three brands of probe wipes effectively removed food soil under both test conditions. Following contamination with the fluorescent soil, probes appeared visually clean however, examination under a blacklight revealed level 3 (medium soil) contamination on two of the three probes cleaned with Wipes Plus brand wipes.

Significance: The use of a fluorescent gel was successful for the evaluation of probe cleaning effectiveness and revealed cleaning deficiencies that were not visible to the unaided eye. This method is recommended as an evaluation tool for food service workers to develop proper technique for cleaning temperature probes between uses for the reduction of microbial contamination and the prevention of cross contamination.

P3-237 Comparing 'Perfect' Food Code Directed Hand Washing Frequency and Technique to Natural Behaviors – What are the Natural Hand Hygiene Behaviors of Retail Food Handlers?

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Introduction: Retail food handlers do not always wash hands with soap and water (HW), or HW 'successfully' as regulated by the Food Code (FC). However, there has yet to be a study directly comparing 'perfect' FC triggered HW with that of naturally occurring HW performance by retail food handlers.

Purpose: Characterize natural HW behavior of retail food handlers compared to those who are guided to near-perfect HW performance based on FC triggers.

Methods: Participants with at least 6 months retail food handling experience (n=38) were instructed to prepare ground beef tacos and salads topped with grilled chicken in research kitchens (NCSU IRB Protocol 21056). Participants were separated into two treatment groups 1) FC-triggered HW (FC-HW; n=20), and 2) those who were instructed to routinely prepare meals as they would at work (Behavior-HW; n=18). Following meal preparation, recorded videos were coded and analyzed for hand washing behaviors.

Results: When participants received instruction on when and how to HW based on FC triggers (FC-HW), an average of 8 HW attempts were required (range 6-16 times). FC-HW participants performed a HW and were successful 94.43% and 99.45% of the time, respectfully. Contrastingly, the Behavior-HW group (no HW instruction) were required to HW an average of 13 times (range 10-19 times), only attempted a hand wash 30.97% of the time, and were only successful 30.79% of the time. The most common reasons for the Behavior-HW group to fail performing a correct HW were not scrubbing hands for 10 or more seconds, not wetting hands prior to scrubbing, and not rinsing hands after scrubbing.

Significance: This study demonstrates that retail food handlers are not likely to perform a HW step as frequently as they should, and when they do, it is not performed correctly.

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P3-238 Effects of Different Hygiene Interventions on Hand Contamination during Meal Preparation in a Simulated Retail Food Setting

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Introduction: Alcohol-Based Hand Sanitizers (ABHS) are not a substitute for handwashing (HW) in retail food establishments per the FDA Food Code (FC). However, comparative studies of various Hand Hygiene Interventions (HHI) in these settings have not been done.

Purpose: To determine the impact of various HHI on the bacterial load on hands after meal preparation in a simulated retail food preparation setting.

Methods: Ground beef and chicken tenderloins were inoculated with 10^9-10^{10} CFU of GFP-tagged *Escherichia coli* DH5-α. Participants with at least 6 months food handling experience (n=85) were assigned to one of five HHI treatment groups: (1) no HHI (Control, n=20); (2) FC-triggered HHI with HW only (FC-HW; n=20); or (3) FC-triggered HHI substituting ABHS for HW (FC-ABHS; n=20). The two remaining groups were asked to routinely prepare meals with (4) access to soap and water only (Behavior-HW; n=18); or (5) access to soap, water and ABHS (Behavior-HW+ABHS; n=7) (NCSU IRB #21056). Hand rinsates (300ml/hand pair) were collected post-experimentation using the glove juice method and enumerated for *E. coli* by direct plating and membrane filtration on selective media.

Results: No significant difference was observed for prevalence of hand contamination between groups (P=0.645). Significantly lower $\log_{10}$ CFU of *E. coli* were recovered, relative to the no HHI Control (4.6±1.3), for FC-HW (2.6±1.1, P<0.001), FC-ABHS (3.4±1.5, p=0.015), Behavior-HW (3.1±1.2, P=0.002), and Behavior-HW+ABHS (2.6±0.8, P=0.002). However, no differences in *E. coli* concentration were observed between groups implementing HHI (P>0.05).

Significance: The concentration of *E. coli* on hands of retail food workers was lower (by 1.2-2.0 $\log_{10}$ CFU) after implementation of any type of HHI, each being roughly equivalent. Future studies should investigate the utility and effectiveness of ABHS as a potential alternative to HW during situations where heavy soiling of hands is not a factor.

P3-239 The Evaluation of Facilities and Hygiene Prerequisites in the National School Nutrition Programme in South African Schools

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Introduction: The prevalence of poverty has increased in recent times, especially in rural areas of developing countries and thus increasing food insecurity. The South African Department of Basic Education's National School Nutrition Programme (NSNP), as part of an Integrated Nutrition Programme (INP), was introduced to reduce food insecurity and the child mortality rate. Even though the Programme has seen some success, it also faces challenges regarding food safety.

Purpose: The study aims to determine the extent to which infrastructure, facilities and hygiene prerequisites have been implemented in schools participating in the NSNP within the Mangaung Metropolitan.

Methods: This descriptive study examined adherence to prerequisites related to food-preparation facilities and hygiene operating guidelines at 98 randomly selected schools using a customized inspection checklist. The study highlighted challenges facing the NSNP at these schools, such as inadequate and poorly maintained infrastructure, food preparation facilities, and a lack of storage facilities.

Results: The situation is aggravated by deficient adherence to hygiene operating requirements such as appropriate facilities for water storage, managing hazardous substances and pest control. The lack of these requirements increases the risk of financial loss due to reducing the shelf-life of foodstuff, food contamination and the outbreak of foodborne diseases. The non-adherence to food safety requirements portrayed in this study has little to do with food handlers' attitudes but rather as the result of a lack of resources necessary for food safety, lack of maintenance of the vandalized equipment and facilities and inadequate initial and continuing training.

Significance: The results show that although the focus was given to basic infrastructure, such as location and structures, other requirements, such as maintenance of such structures, food preparation equipment and hygienic operating prerequisites, still needed attention. This study provides the Prerequisites that are still required and paves the possibility of implementing food safety systems such as HACCP.

P3-240 Microbial Profile of Food Handlers' Hands before and after Hand Washing

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❖ ***Developing Scientist Entrant***

Introduction: Good practice of hand washing is a pre-requisite in the control of microbial safety and reduce cross-contamination in the food industry while the total plate count (TPC) method is not considered a good parameter to measure the hand hygiene of food handlers.

Purpose: This study investigates the microbial profile of food handlers' hands during hand washing, aiming to propose a better indicator of hand hygiene.

Methods: 28 food handlers working at NUS canteens were recruited, and their hands were swabbed before and after hand washing with non-disinfectant hand soap. Samples were enumerated/detected with plate count agar (PCA), violet red bile glucose agar (VRBGA), Baird-Parker agar (BPA) and Methicillin-resistant *Staphylococcus aureus* agar (MRSA).

Results: After hand washing, significantly higher reductions in bacteria enumeration were observed using VRBGA (0.78 ± 0.85 log MPN/hand reduction) than using PCA (0.21±0.89 log CFU/hand reduction, P < 0.05), BPA (0.02 ± 0.78 log CFU/hand reduction, P < 0.05) or MRSA (0.26 ± 0.72 log CFU/hand reduction, P < 0.05). Interestingly, a number of the PCA enumerations were increased after hand washing, presumably due to the reduction of antagonistic microflora over *S. aureus* and thus more *S. aureus* were allowed to grow on PCA plates (proposed based on morphology observation and phenotypic confirmation at an ad-hoc base). As a preliminary proof, the BPA count after hand washing is negatively correlated to PCA reduction (R^2 = 0.2196, P = -0.45). Last, the common presence of MRSA was observed on the hands of the participants both before (26/38 positive) and after hand washing (19/38 positive).

Significance: This study serves as a base-line microbial investigation of food handlers' hand washing events. It is suggested that VRBGA might be more suitable to indicate the hand hygiene than PCA. VRBGA is mainly used to enumerate Enterobacteriaceae, an important hygiene and safety indicator in food microbiology.

P3-241 Evaluation of the Effect of Sodium Hypochlorite Washing and Hot-Air Drying to Reduce Coliform in Barley Sprout Processing Plant-Case and Improved the Drying Efficiency

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Introduction: Barley sprouts have received much interest as functional foods in many countries. However, its product is often contaminated by coliform and *Escherichia coli* originated from soil.

Purpose: This study investigated the effect of sodium hypochlorite washing and hot-air drying on the reduction of coliform during barley sprouts processing in plant-scale and improved the drying efficiency.

Methods: While washing 400 kg of barley sprouts with 100ug/kg sodium hypochlorite water, we took 100 g sample every 100 kg washing and analyzed total aerobic bacteria, coliform and *E. coli* counts. Then, 100g samples of each 100kg (fresh weight) barley sprout dried at 60 °C for 18h were collected to analyze microbial counts. To improve the drying efficiency, we changed the drying temperature to 70, 75, 80°C and analyzed microbial counts, chromaticity and functional component content (saponarin and policosanol).

Results: As a result of analyzing the washed samples, coliforms were detected at a level of 2.1 ± 1.1 log CFU/g in the first sample and 3.3 ± 0.3 ~ 0.4 log CFU/g in the subsequent samples. In the dried sample, coliforms were detected as 3.0 ~ 4.0 log CFU/g, so there was no effect of reducing coliform by hot air drying at 60°C. When it was dried at 70°C, coliform was reduced by more than 4 log CFU/g($p<0.05$) without change in color and functional components. At the higher temperatures, coliform was decreased faster then at 70°C ($p<0.05$) but it occurred a large change in color.

Significance: These results reveal that 100ug/kg sodium hypochlorite washing and 60°C hot-air drying, which is used as critical control point (CCP) in barley sprout processing, were not effective in control of microbiological hazards. In order to increase the effect of reducing coliform and minimize quality degradation, it is appropriate to set the drying temperature to 70°C.

P3-242 The Effect of Antimicrobial Use over Time on the Properties of Polyethylene Terephthalate (PET) Commonly Found in Foodservice Establishments

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❖ ***Developing Scientist Entrant***

Introduction: During the COVID-19 pandemic many foodservice/retail establishments increased their usage of antimicrobial products. Repeated use of antimicrobials could cause physical and chemical changes to surface materials. These changes could result in minute crevices that can harbor foodborne pathogens.

Purpose: We characterized physical and chemical changes to polyethylene terephthalate (PET) after simulated long-term use of antimicrobial products.

Methods: Virgin samples of PET (252 coupons) were tested. Coupons were exposed to an accelerated antimicrobial treatment using a Gardner scrub abrasion tester to simulate use after 6, 12, and 24 months (200, 400, and 800 swipes, respectively). Two antimicrobials—quaternary ammonium chloride and bleach—were used at sanitizer and disinfectant levels. Coupons were assessed using six testing methods—confocal microscopy, optical microscopy, tensile strength, change of mass, contact angle measurement, and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) — before and after accelerated antimicrobial treatment. Statistical analysis was completed with JMP using two-way ANOVA.

Results: Spectral changes were observed over time using FTIR-ATR between treated and untreated PET coupons. Contact angle measures decreased (surface energy increased) when treated with bleach and quaternary ammonium chloride over time ($p<0.0001$), which correlated with the degree of change in roughness and other topographic features, such as scratches and stress whitening, characterized using optical and confocal microscopy. After prolonged exposure to antimicrobial products, tensile properties of PET coupons changed ($p<0.0001$). Change of mass was not affected by any of the antimicrobial products with time (($p<0.0001$).

Significance: Antimicrobial use over time can cause physical and chemical changes to PET. These changes could possibly result in increased colonization of pathogens and/or creating better opportunities for attachment or biofilm formation of pathogens to surfaces, making it more difficult to effectively clean then treat a surface constructed from PET.

P3-243 Efficacy of Chlorine, Chlorine Dioxide, Peroxyacetic Acid, Steam and Silver-Dihydrogen Citrate in Controlling *Escherichia coli* Biofilms on Harvesting Bins and Picking Bags

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❖ ***Developing Scientist Entrant***

Introduction: Tree fruits have been implicated in foodborne outbreaks and recalls making producers reevaluate efficacy of sanitation practices for food contact surfaces used during harvesting and handling. When tools, bins, and containers are not effectively sanitized, microbial communities may develop. *Escherichia coli* (*E. coli*) can form biofilms on a variety of surfaces. The characteristics of these surfaces play an important role in the strength of attachment, growth, and ability to be disrupted by sanitizers.

Purpose: This study compares the efficacy of commercially available sanitizers in reducing *E. coli* biofilms on surfaces commonly used during tree fruit harvesting.

Methods: Three strains of STEC were combined and grown for 96 hours to form biofilms in a CDC reactor at 25± 2°C on high density polyethylene plastic (HDPE), wood, or nylon. Mature biofilms were exposed to chlorine dioxide (400 ppmv) for 24 hours, or free chlorine (500 ppm), peroxyacid (500 ppm), steam (75 psi) and silver-dihydrogen citrate (4%) for 1 or 2 minutes. Coupons were neutralized, and cells detached through sonication and vortexing, then enumerated. Treatment times were randomized across rods within the bioreactor, and replicated six times with positive controls. Results were considered significant at $P< 0.05$.

Results: Chlorine dioxide was the most effective treatment in controlling *E. coli* biofilms compared to other interventions studied. On nylon, 2-minute peroxyacid exposure time resulted in higher reduction on nylon, but HDPE and wood surfaces had similar levels of reduction at both exposure times ($P>0.05$). Steam caused significant reductions on all surfaces ($P<0.05$). Contact time did not impact efficacy for steam or silver-dihydrogen citrate within sanitizer type ($P>0.05$). There was no difference in reduction across treatment time on HDPE or nylon surfaces ($P>0.05$) for free chlorine treatment.

Significance: Understanding how surface characteristics impact sanitizer efficacy can lead to better management practices for food contact surfaces used during tree fruit harvesting.

P3-244 Quantifying Cleanliness of Food Contact Surfaces Using Conductivity of Total Dissolved Solids

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❖ ***Developing Scientist Entrant***

Introduction: The concept of a "clean surface" in the food industry is often used interchangeably with a subjective term, "visually clean". Thus, methodologies to quantify cleanliness of a surface need to be developed.

Purpose: The study tests the viability of using conductivity of total dissolved food particles as a quantification for cleanliness of a surface.

Methods: Table salt and soy protein powder isolate were used due to being both water soluble and conductive. For the control, powders (0.00, 0.05, 0.10, and 0.15 g) were added to 30 mL of deionized water in a glass beaker and agitated with a sonicator until fully dissolved. For the swabbing method, the same powder amounts were distributed onto stainless steel coupons (8 x 8 cm) and collected using a sponge swab probestick. Thereafter, sponge samples were agitated for 120 s in a masticator and the resulting 30 mL solution was poured into glass beakers. Conductivity of each solution was mea-

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sured using a conductivity probe in triplicate. Conductivity for concentrations gathered from control and swabbing methods were compared in terms of 95% confidence t-test and correlation. Regression lines for each data set were compared using analysis of covariance.

Results: Conductivity was significantly greater at all concentrations for samples taken with the sponge swab (P < 0.01). The swab samples at 0.00 g application showed statistically significant conductivity, which indicates unexpected interactions between the sponge and solutions (R^2=0.61 for salt; R^2=0.87 for soy protein). Analysis of covariance found that powder type, use of swab, and their interactions had a significant impact on the conductivity curve.

Significance: Using a sponge swab significantly alters the conductivity response. Thus, swab methods should be further researched for improvement.

P3-245 Investigation of Hydrophobic Properties of Silane-Treated Wood Through Micro-Topographical Analysis

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❖ ***Developing Scientist Entrant***

Introduction: Wood is still utilized in fresh fruit and vegetable production, post-harvest handling, and retail. Silane treatment of wood has been demonstrated to be a useful method of producing antifouling properties in wood, although very little is known regarding its mode of action in repelling water-suspended microbes.

Purpose: The purpose of this study was to characterize the micro-topographical changes of two wood species (pine, oak) after a fluoroalkyl-silane antifouling treatment, to better understand the interactions between silane with wood and the consequent repulsion of bacterial foodborne pathogens.

Methods: Pine and oak square-cut surfaces measuring 4 cm^2 were submerged in 1% (w/w) heptadecafluoro-1,1,2,2-tetrahydrodecyl trichlorosilane to yield antifouling properties on treated surfaces or left untreated (control). Wood surface morphologies were then analyzed using atomic force microscopy (AFM) to determine pre- and post-treatment roughness ratio, effectively the relationship between the actual and projected surface area of a surface. Silane deposition on wood block surfaces was also visually characterized using scanning electron microscopy (SEM).

Results: One-way analysis of variance of least square means determined that pine AFM images (25 μm^2; n=50) reported a statistically significant difference (P=0.0005) in roughness ratio between treated and untreated samples. Similar results were observed with oak samples (25 μm^2; n=50). SEM analysis indicated interstitial deposition of silane between wood fibers of pine, versus primarily a superficial deposition on oak.

Significance: Data gathered regarding the microscopic surface characteristics contributed to the understanding of water repulsion mechanisms by silane treatment on pine and oak samples. Microscopic imaging demonstrated that the resulting super-hydrophobicity may not only be a function of the altered surface chemistry of the treated wood, but also of the consequent shifts in surface roughness produced by such treatment.

P3-246 Quantitative Analysis of Surface Thermal Uniformity on Stainless Steel during Superheated Steam Sanitation Using Thermal Image Processing Techniques

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❖ ***Developing Scientist Entrant***

Introduction: Superheated steam is an emerging technology for the sanitation of food processing environments. However, there is limited data available on treatment uniformity during pilot-scale superheated steam sanitation.

Purpose: The objective of the study was to quantitatively evaluate the thermal distribution on stainless steel surface during superheated steam using thermal image processing that aid in the validation of the sanitation efficiency of superheated steam.

Methods: For the quantitative analysis of surface temperature, the thermal camera (IR281, PerfectPrime, North Bergen, NJ) was calibrated with a small stainless steel coupon (5cm×5cm×1cm) at various temperatures (25°C-250°C). Superheated steam treatment was conducted using a pilot-scale superheated steam generator (SaniZap-600-40240, Bayzi, Cincinnati, OH) on a stainless-steel coupon (30cm×30cm×1cm). Distance between nozzle and treatment surface varied between 2-5cm. The surface temperature distribution during treatment was recorded using the calibrated thermal camera. The successive thermal images were processed by the following steps: image acquisition, perspective control, segmentation, and analysis. All experiments were triplicated.

Results: The thermal camera was calibrated to monitor the surface temperature of stainless steel (R^2=0.99 at 0.23 emissivity). As the distance between nozzle and treatment surface decreased from 5 cm to 2 cm, the surface temperature at the center increased from 157.6±1.7°C to 245.6±3.9°C. The surface area where the temperature is above 125°C increased from 15.4±0.8cm^2 to 35.7±1.3cm^2 as the distance decreased from 5cm to 2cm. It is worth to point out that the surface temperature decreased away from area of impingement. For example, the surface temperature was below 100°C at 5.3±0.1cm and 3.6±0.1cm away from the treatment point at 2cm and 5cm distance, respectively. More research is needed to understand effective sweeping techniques that would result effective sanitation of larger surfaces.

Significance: The quantitative analysis of surface thermal uniformity developed in this study can be used to validate the sanitation efficacy of superheated steam.

P3-247 Factors Affecting the Growth and Attachment of *Listeria monocytogenes* on Food Contact Surfaces

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Introduction: Food contact surfaces have been implicated to be one of the sources of *Listeria monocytogenes* contamination in most of the food borne disease outbreaks. The attachment and biofilm forming ability of microorganisms have been found to be influenced by various factors such as microbial strain and the temperature and pH of the environment.

Purpose: This study investigated the effect of environmental temperature and lineage (Lineage I and Lineage II) of *Listeria monocytogenes* on attachment and biofilm formation on different food contact surfaces such as Silicon Rubber, PVC, HDPE, LDPE, PET, and stainless steel.

Methods: Coupons (1.5cm^2) of six different food contact surfaces (Silicon Rubber, PVC, HDPE, LDPE, PET, and stainless steel) were immersed in 2ml of 1:10 diluted apple juice contaminated with 7 log CFU/ml *Listeria monocytogenes* (serotype 4b- Lineage I and serotype 1/2a -lineage II). Listeria levels on the coupons after removing the unattached cells at 4°C and 25°C were evaluated. Samples were plated on TSBYE and incubated at 37°C for 24 h for enumeration.

Results: After 7 days of immersion in contaminated apple juice, *Listeria monocytogenes* on each coupon were attached up to 5.06 - 5.27 Log CFU/coupon. Listeria levels were significantly higher (P<0.05) at 25°C for both lineages. The type of surface matrices has no effect on Listeria levels, except for silicon rubber (5.11 Log CFU/coupon), which was significantly different than Stainless steel (5.17 Log CFU/coupon). A significant effect of lineage type was observed with lineage II resulting in higher levels of Listeria counts than lineage I at both temperatures.

Significance: Left over fruit juices on food contact surfaces may promote the growth and attachment of environmental pathogens such as *Listeria monocytogenes* and increase risk of cross contamination during processing.

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P3-248 Use of UV-C and Lactic Acid in Slaughterhouses and Meat Processing Plants to Reduce Fungi

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Introduction: In prior studies, fungi such as *Cladosporium* and *Penicillium* were frequently detected in livestock production. Therefore, it becomes important to develop an anti-fungal treatment which is economical and easy to use for the workers

Purpose: In this study, we investigate the use of UV-C and lactic acid and their combination in slaughterhouse and meat processing plants.

Methods: The reduction of fungi was demonstrated on a lab scale to optimize the UV-C irradiation and the time of lactic acid treatments. Five strains were inoculated on a stainless steel chip, and the UV-C irradiation time was optimized for fungi reduction. Then, in the lactic acid test, 1 ml of 4% lactic acid was sprayed, and fungi reduction was confirmed after 0.5, 1.3, 6, and 12 h of treatment. A method combining UV-C and lactic acid was then optimized to investigate the anti-fungal treatment on the tools and surfaces in the slaughterhouse and meat processing plant.

Results: All five molds were significantly reduced ($p<0.05$) by at least 2.1 log CFU/cm^2 after UV-C irradiation, compared to the control. When treated with lactic acid, all of the molds, except for *P. commune*, were not detected (ND = 10) after 6 h. Fungi were significantly ($p<0.05$) reduced from 0.7 to 3.4 log CFU/cm^2 after spraying 1 ml of lactic acid (4%) and irradiating 10.8 mJ/cm^2 of UV-C treatment at the slaughterhouse and meat processing plant (shackles, knives, working board, conveyer belt, saw blade). More than 99% of the tools and surfaces were successfully treated, except in the case of shackles, where the surface was curved, rendering difficulty in applying the anti-fungal agent.

Significance: Lactic acid and UV-C will be able to hygienically manage tools used in small slaughterhouses and meat processing plants.

P3-249 Inactivation of *Listeria monocytogenes* on Inert Surfaces Using High-Intensity Blue Light

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❖ ***Developing Scientist Entrant***

Introduction: *Listeria monocytogenes* in the environment poses a major concern to the food industry. Current sanitation programs would benefit from additional surface interventions such as antimicrobial blue light (aBL) to inactivate biofilms on food contact surfaces. We previously reported that low-intensity aBL inactivated *L. monocytogenes* biofilms using low-intensity lamp treatments.

Purpose: This study was conducted to assess the viability reduction of *L. monocytogenes* dry cells and biofilms on stainless steel (SS) coupons using high-intensity aBL lamps.

Methods: A 5-strain cocktail of *L. monocytogenes* was used to grow 48 h biofilms on SS coupons using petri plates in batch cultures and in a Drip flow biofilm reactor (DFBR) at room temperature. Dried cells were prepared by placing and drying cultures on coupons. Coupons with biofilms and dried cells (6 - 7 Log CFU) were exposed to aBL (405 nm wavelength) using high-intensity lamps (emissions of 1,600 and 3,500 mW/cm^2, respectively) at different doses at different distances. Exposure dosage was calculated by multiplying irradiance (mW/cm^2) by time (s). Treated coupons were processed by sonication and counts were measured by standard plating on tryptic soy agar, followed by incubation at 37 °C for 24-48 h. Independent experiments were conducted at least twice. Statistical analyses were performed using Students' t-test.

Results: The viability of *L. monocytogenes* biofilms grown in batch and in DFBR was reduced by 3.1 log CFU and 1.9 ± 0.04 Log CFU/coupon at different exposure times and dosage of 4 h (2,000 J/cm^2) and 2 h (828 J/cm^2), respectively, compared to controls ($p<0.05$). When a meter of separation from the 3,500 mW/cm^2 lamp was used, more than 1.5 CFU/cm^2 dried cells were inactivated after 24 h. The combinations of sanitizers with aBL were also investigated.

Significance: The wide use of high-intensity lamps for other applications offers the potential to optimize the exposure treatment with aBL.

P3-250 Comparative Study of the Susceptibility to Blue Light Inactivation of Foodborne Pathogens and Spoilage Bacteria

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❖ ***Developing Scientist Entrant***

Introduction: Antimicrobial Blue Light (aBL) in the visible spectrum of 400–470 nm has been reported to be a potential intervention technology for treating surfaces. Multiple microbial species have been described to be susceptible to aBL. However, a systematic side-by-side comparison for efficacy of aBL against food-related bacteria is largely missing in the literature.

Purpose: This study was conducted to determine the broad-spectrum effect of aBL against selected foodborne pathogenic and food spoilage bacteria.

Methods: Cocktails of *Listeria monocytogenes* (Lm), *Salmonella*, *Cronobacter* and *Pseudomonas* were inoculated in tryptic soy broth (TSB), phosphate buffered saline (PBS), and on stainless steel (SS) coupons. Coupons were dried overnight. Viable cell count was determined before and after exposure to 405 nm aBL at 4 °C and room temperature. Time of exposure was based on the target irradiation dose, 1,500 and 1,700 J/cm^2 for liquid cultures and dry cells, respectively. Dry cells were resuspended by sonication before plating. Counts were calculated by standard plating on tryptic soy agar (TSA) after incubation at 37°C for 24–48 h. Statistical analyses were conducted using ANOVA.

Results: Viable counts of all species were reduced > 6 Log CFU/ml when incubated in TSB during aBL treatment, but in PBS viability decreases of < 2.1 Log CFU/ml were observed with the exception of *Pseudomonas. Pseudomonas* was the most susceptible bacteria to aBL at all conditions, inactivated more than 6 Log CFU/ml in liquid and on coupons. The viability of *Cronobacter* and *Salmonella* dried cells was only 1.5 Log CFU less than controls after treatment. In contrast, viability reductions of *L. monocytogenes* and *Pseudomonas* dried cells were > 3 Log CFU ($p<0.05$). No significant temperature effect was determined at any experimental condition. ($p>0.05$).

Significance: These findings suggest that the susceptibility to aBL may be variable among different food-relevant bacterial species.

P3-251 Ozonated Water Use for Operational Sanitation during Beef Fabrication

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❖ ***Developing Scientist Entrant***

Introduction: Sanitation procedures during beef fabrication can be critical for safety as cross-contamination can occur on equipment, utensils, and surfaces.

Purpose: To assess the efficacy of an ozonated water (Biosafe™) system as an antimicrobial intervention on fabrication surfaces.

Methods: 70 samples were collected from the chuck fabrication line in the morning, 2 hrs into processing and at the mid-day break, 4 hrs into production, divided in 4 repetitions. MicroTally™ cloths were used to sample cutting boards (CB) units of 0.5m^2; MicroSnap™ swabs were used to sample knives (20 cm^2) before and immediately after Biosafe™ application. Aerobic plate counts (APC), Enterobacteriaceae (EB), and *Escherichia coli* (EC) counts were determined using the TEMPO® system for MicroTally™ samples, and the EnSURE™ Touch system was used for MicroSnap™ swabs.

Results: The following values are expressed in LogCFU/cm^2; APC counts in the morning were 3.47 before and 2.64 after for CB ($p>0.05$). For knives, 2.63 before and 2.15 after ($p>0.05$). At mid-day, 3.77 before and 3.1 after for CB ($p<0.05$). For knives, 2.13 before and 2.06 after ($p>0.05$). EB counts in the morning were 3 before and 1.73 after for CB (<0.05), 1.42 before and 2.06 after for knives ($p>0.05$). At mid-day, values were 2.84 before and 2.18 after for

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CB (p>0.05), 0.78 before and 0.84 after for knives (p>0.05). Finally for EC, morning values were 0.44 before and 0 after (p<0.05) for CB, 0.4 before and 0.44 after for knives (p>0.05). Mid-day values were 0.71 before and 0.27 after for CB (P<0.05), 0.44 before and 0.4 after for knives (p>0.05).

Significance: The use of ozonated water reduced microbial indicators in CB. However, for the knives the reduction was not significant, meaning that increased concentration, contact time, or another intervention is necessary to sanitize knives throughout the day. Therefore, the Biosafe™ intervention is recommended to improve the cleaning of CB.

P3-252 Chemical Sanitizer's Effectiveness to Eliminate Multispecies *Escherichia coli* O157:H7 and Spoilage Bacteria on Food Contact Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Despite compliance with cleaning and sanitation standards, the food industry faces food safety and quality issues related to bacterial persistence on food contact surfaces.

Purpose: To evaluate the effectiveness of biocides when applied to mature multispecies biofilms formed by STEC and spoilage bacteria.

Methods: *Carnobacterium piscicola* + *Lactobacillus bulgaricus* biofilms were formed on polyurethane (TPU) and stainless steel (SS) surfaces by inoculating each strain (10^6 CFU/ml) on LB-NS broth and incubating 6 days, at 10°C and 25°C (n=864). Biofilms were washed 3 times with BPB. *E. coli* O157:H7 was added (10^3 CFU/ml) to the preformed biofilms and incubated for 6 days. Biofilms were washed 3 times and stored under wet (60–90% RH) and dry (20–50% RH) conditions for 6, 30, and 60 days at 10°C and 25°C. Coupons exposed to detergent (scrubbed or not scrubbed), rinsed and treated with quats, sodium hypochlorite (SHypo), sodium hydroxide (SHyd), hydrogen peroxide (HyP), peroxyacetic acid (PeroA) or BioDestroy®. The bacterial reduction was calculated.

Results: Quats and SHyd were less effective (P < 0.001) in wet and dry conditions at 25°C when SS was not scrubbed. Biofilms on TPU at 25°C displayed 4.5 log10 CFU/cm^2 survival to biocides regardless of the cleaning method (P <.0001). The best reductions on TPU coupons at 25°C were BioDestroy® (8.2 log10 CFU/cm^2), followed by SHypo (7.6 log10 CFU/cm^2), HyP (6.5 log10 CFU/cm^2), SHyd (4.8 log10 CFU/cm^2) and quats (3.7 log10 CFU/cm^2) (P <.0001). Biofilms on SS and TPU unscrubbed at 10°C showed resistance to quats (P <.0001). Dry biofilms (SS and TPU) were overall more resilient (P <.0001). Single species STEC and spoilage biofilms showed resistance only to sodium hypochlorite at 10°C and 25°C (P <.0001).

Significance: This research shows that proper biofilm mechanical disruption during cleaning is critical to reducing O157 and spoilage microorganisms on food contact surfaces.

P3-253 Effect of Disinfectants on New and Mature Shiga Toxigenic *Escherichia coli* and Spoilage Multispecies Biofilms Formed at Different Temperatures.

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❖ ***Developing Scientist Entrant***

Introduction: Food-borne pathogens and spoilage bacteria can adhere to the surface of equipment, resulting in the formation of multispecies biofilms. Complex interactions among microorganisms may affect the susceptibility of biofilms to disinfectants.

Purpose: To investigate the effectiveness of disinfectants on multispecies biofilms formed by STEC and spoilage bacteria.

Methods: Three multispecies biofilms combinations T1: *Carnobacterium piscicola* + *Lactobacillus bulgaricus*, T2 *Comamonas koreensis* + *Raoultella terrigena* & T3 *Pseudomonas aeruginosa* + *C. koreensis* were formed (10^6 CFU/ml) for 6 days on polystyrene 96-wells microplates containing LB-NS at 10°C and 25°C (n=1440). Biofilms were washed 3 times with BPB. , STEC individual strains 10^3 CFU/ml (O157:H7, O145:H2 & O103:H2) were added to the preformed biofilms, incubated for 6 days, and washed 3 times. Biofilms (0 and 30 days old) were treated with quats, sodium hypochlorite (SHypo), sodium hydroxide (SHyd), hydrogen peroxide (HyP), peroxyacetic acid (PeroA) and BioDestroy®organic peroxy-acid. Biofilm eradication concentration was calculated as the lowest antimicrobial concentration that prevented the bacteria's visible growth.

Results: Spoilage bacteria on days 0 and 30 within T1, T2 and T3 showed more resistance to SHyd (30%), followed by quats (23.25%), HyP (15.41%), SHypo (9.70%), and BioDestroy® (3.42%) (*P* <.0001). STEC combined with T1, T2 and T3 showed more resistance to quats (23.91%), followed by HyP (19.57%), SHypo (18.12%), SHyd (16.67%), and BioDestroy® (4.35%) (*P*<.0001) at 10°C and 25°C. O157:H7-R5O8, when present with T2 and T3, showed resistance to quats and sodium hypochlorite (*P*<.0001). O157:H7 and O103:H2, combined with T1, T2 and T3, exhibited the highest resistance to biocides, while O145:H2 (weak biofilm former) exhibited the least (*P*<.0001). STEC and spoilage single-species controls showed survival for SHypo at 10°C and 25°C (*P* <.0001).

Significance: Quats and SHyd were least effective on multispecies biofilms, while organic BioDestroy® was the most effective sanitizer (*P*<.0001). Comparatively, multispecies biofilms exhibited higher resistance to disinfectants than single-species biofilms.

P3-254 Effects of Different Hand Hygiene Interventions on Cross-Contamination of Kitchen Surfaces during Meal Preparation

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❖ ***Developing Scientist Entrant***

Introduction: The FDA Food Code (FC) does not recognize Alcohol Based Hand Sanitizers (ABHS) as an alternative to handwashing (HW), however ABHS efficacy in food handling settings has not been systematically studied.

Purpose: To monitor bacterial cross-contamination from raw meat to kitchen surfaces after the application of various hand hygiene interventions (HHI).

Methods: Ground beef and chicken tenderloins were inoculated with 10^9-10^{10} CFU/g of *Escherichia coli* DH5-α. Participants with at least 6 months of food handling experience (n=85) were assigned to one of five HHI groups (NCSU IRB 21056). For three groups, participants were asked to routinely prepare meals with (1) no HHI (Control, n=20); (2) access to soap and water only (Behavior-HW; n=18); or (3) access to soap, water and ABHS (Behavior-HW+ABHS; n=7). Remaining groups were instructed by researchers on when and how to perform HHI based on FC triggers with (4) HW only (FC-HW; n=20); or (5) ABHS only (FC-ABHS; n=20). Environmental swabs were collected from the first five surfaces touched after handling raw meat and enumerated for *E. coli*.

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Results: Differences in frequency of surface contamination were observed between treatment groups (*P*<0.001), with the no HHI Control [73% (145/200)] having significantly higher frequency compared to the other groups (*P*<0.001). When compared to FC-HW [15% (29/200)], no significant differences in surface contamination frequency were observed for FC-ABHS [8% (16/200), *P*= 0.168], Behavior-HW [10% (18/180), *P*=0.558], or Behavior-HW+ABHS [20% (14/70), *P*=0.168]. Of the surfaces that were contaminated, no significant differences were observed in log concentration of *E. coli* between treatment groups (*P*=0.424).

Significance: HW and ABHS interventions produced similar frequency of kitchen surface cross-contamination events, and both resulted in significantly lower contamination frequency compared to the no HHI control group. Future work investigating the combined use of HW and ABHS as HHI in retail kitchens is needed.

P3-255 Impact of Gas Ultrafine Bubbles on the Efficacy of Antimicrobials for Eliminating Fresh and Aged *Listeria monocytogenes* Biofilms on Dairy Processing Surfaces

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❖ ***Developing Scientist Entrant***

Introduction: Ultrafine bubble (UFB) technology is a novel concept in food processing that has the potential to enhance the potency of commonly used antimicrobials to eliminate biofilms.

Purpose: This study investigated the impact of incorporating gas [air, carbon dioxide (CO_2) and nitrogen (N_2)] UFBs on the potency of chlorine (Cl_2; 50, 100, and 200 ppm) and peracetic acid (PAA; 20, 40, and 80 ppm) antimicrobial (AM) solutions against fresh (3 day) and aged (30 day) *Listeria monocytogenes* biofilms on polypropylene, silicone, and stainless-steel surfaces.

Methods: The *L. monocytogenes* biofilms were statically grown on polypropylene, silicone, and stainless-steel coupons (2.54 × 7.62 cm) at 25°C for 3 days or 30 days, by immersing in *L. monocytogenes* inoculated brain heart infusion (BHI) broth. On day 3 or 30, the coupons were treated by submerging in AM solutions with and without UFBs for 1 minute, and then were swabbed into Dey-Engley neutralizing broth and enumerated on BHI agar. The log reductions for the respective antimicrobial treatments were calculated by subtracting post-treatment biofilm bacterial populations from untreated biofilm bacterial populations.

Results: Incorporation of CO_2 UFBs in AM solutions resulted in significantly greater log reductions (2.4-3.9 CFU/cm^2) of fresh and aged *L. monocytogenes* biofilms on polypropylene, silicone, and stainless steel compared to AM solutions without UFBs on the respective surfaces (1.4-2.9 CFU/ cm^2). This study also demonstrated that the incorporation of 200 ppm Cl_2 resulted in significantly greater log reduction of *L. monocytogenes* biofilms on fresh and aged polypropylene, silicone, and stainless-steel surfaces compared to 50 ppm Cl_2, 20 ppm PAA, and 40 ppm PAA.

Significance: This study demonstrated that using CO_2 UFBs in Cl_2 and PAA solutions significantly increase their potency against fresh and aged *L. monocytogenes* biofilms on polypropylene, silicone, and stainless-steel surfaces.

P3-256 Disinfectant Type and Contact Time Impact Disinfectant Towelettes Efficacy over Large Surface Areas

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❖ ***Developing Scientist Entrant***

Introduction: Disinfectant towelettes are increasingly used to decontaminate surfaces due to convenience and reliable performance.

Purpose: The objectives of this study were to (i) evaluate the efficacy of selected disinfectant towelettes on hard, non-porous surfaces contaminated with *Candida auris, Staphylococcus aureus,* or *Pseudomonas aeruginosa* and (ii) determine if a one-minute contact time results in a significant reduction of pathogens compared to other fixed contact times (30 s, two min, three min, 10 min).

Methods: This study examined the effectiveness of one quaternary alcohol-based, one hydrogen peroxide-based, and three quaternary-based disinfectant towelettes against *C. auris* (ATCC 5001), *S. aureus* (ATCC 6538), and *P. aeruginosa* (ATCC 15442) on hard, non-porous surfaces to determine the fungicidal and bactericidal efficacy of disinfectants under conditions that mimic real-world use.

Results: Contact time significantly impacted the fungicidal efficacy, while bactericidal efficacy was strain dependent among the disinfectant towelettes tested. Hydrogen peroxide-based towelettes showed high fungal and bacterial reduction levels among all three pathogens tested. Whereas, quaternary-based disinfectants had varying results depending on the specific formulation. Regardless of the disinfectant type evaluated for *C. auris*, a 30 s contact time was significantly less effective than one min contact time (p<0.0001), and none of the other disinfection times (two min, three min, 10 min) had a significant difference compared to one minute. Among the two bacterial species tested, only the 30 s and 10 min contact times were significantly different for *P. aeruginosa* (p=0.0129). Whereas, *S. aureus* showed no difference in efficacy among any of the contact times.

Significance: Disinfectant efficacy varies among product types and contact times for selected pathogens. Product type significantly impacts fungal and bacterial reduction on hard, non-porous surfaces. Contact time has a greater effect on disinfectants' fungicidal efficacy than bacteria, highlighting the importance of product selection and adhering to label use guidelines.

P3-257 Best Practices for Allergen Removal via Wet Sanitation Chemistry

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Introduction: Allergen control is highly critical in food sanitation industry and often wet sanitation is used to help monitor soil-build up and chemistry effectiveness on different allergens and surfaces.

Purpose: Compare the ability of multiple chlorinated, caustic, acid-based cleaners and different applications to show their efficacy to remove allergens from surfaces. Confirm post checks using Hygiena® AllerSnap® to check for residual allergen protein after chemistry is incorporated.

Methods: Stainless steel 4x4 sheet swabbed using Hygiena® AllerSnap® for specific allergen to show no existing allergen. 1g allergen specific product [ex. Peanut butter] spread on sheet. Dried 30 mins and removed from surface using specific TACT method. 3 common allergens tested: tree nuts, lactose, gluten. Swabbed again to show positive protein with product. Time range: 1 min, 10 min, 30 min. Action: scrub brush, agitation soak. Chemistry in products: chlorinated foaming, 50% citric, 30% citric, 45% KOH, 50% NaOH, 50% caustic and NaOH blend. Temperature: range from 90°F-120°F depending on PDS for soak. RT was used for scrub brush. Post-TACT method, sheets were swabbed again using AllerSnap® to show cleaning efficacy of chemistry.

Results: Tree nuts were tested with chlorinated foaming (100°F) and 50% citric product (140°F) at different times/applications. Results showed that 1 min agitation soak did not fully remove allergen protein. 5 and 30 min agitation soak removed all tree nut protein residue using both chemistries tested. All times for scrub brush method removed tree nut protein residue at room temp. Results will continue to be obtained with more allergen types and chemistry.

Significance: Permits company to create and utilize a subcomponent of products and program needed by current and future customers. Results will help customers create SSOPs based on types of allergens and equipment used.

P3-258 Relative Performance of Rapid Hygiene Assays Against Allergen-Laden Soils

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Introduction: Rapid hygiene assays are key elements of successful, effective means of verifying cleaning and sanitation processes. The sensitivity and performance of such assays become especially critical when allergen control is nested within food safety plans. The various assay technologies available in the market fundamentally differ in function and design. Yet there are few studies that provide a comparative assessment of these technologies applied to allergen-based soils.

Purpose: This work compares the sensitivity and performance of different rapid assay technologies against food allergen soils including limits of detection.

Methods: We evaluated and compared four major technologies for allergen detection: ATP detection, total adenylate (ATP+ADP+AMP, hereafter denoted as A3), general protein detection, and lateral flow immunoassay (hereafter denoted as ICM). With each method, we examined a broad range of allergens, including egg, soy, wheat, peanut, sesame, shrimp, tilapia and beef. Limits of detections were determined with serial dilutions of allergen soils at a native state and after a denaturation-level thermal treatments. Analysis of variance with an alpha of less than 5% was used as the means of comparing assay and treatment effects with pairwise comparisons completed where appropriate.

Results: For all detection strategies and allergens, thermal treatments had negligible effect on detection sensitivity. Using different assays on ten-fold dilution series of each food product, we found ICM and A3 methods to be superior to other assays. Between these techniques, ICM was the most sensitive for egg, wheat, peanut, and sesame; A3 assay was the most sensitive for soybean; and ICM and A3 were equally sensitive for shrimp and tilapia.

Significance: The management and control of allergen based soils is a significant concern for food processors. Here we present indications that limits of detection for specific food allergens will vary by orders of magnitude based on the technology of the assay selected for each specific assay.

P3-259 Effects of Adaptive Tolerance of Benzalkonium Chloride on *Salmonella* Biofilm Formation

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Introduction: An inappropriate use of benzalkonium chloride (BZK) may provide a sub-lethal exposure resulting in the development of bacterial adaptation.

Purpose: The purpose of this study was to evaluate the adaptive effects of BZK on the biofilm formation of three chicken-isolated *Salmonella* strains, including *S*. Typhimurium ATCC 14028, *S*. Typhimurium s-oola, and *S*. Enteritidis z-2008.

Methods: The minimum inhibitory concentrations (MICs) of BZK against *Salmonella* strains were determined using the microdilution method. Then 20 µL of the culture (~10^8 CFU/mL) from the wells containing ½MIC of the sanitizer was transferred to 180 µL of TSB containing BZK with a final concentration of 1.5 times higher than the previous wells and incubated at 37 °C for 24 h. The transfer procedure was repeated until no growth was observed. The stability of the adaptation was assessed by subculturing the adaptive pathogens in TSB without BZK for 10 generations, followed by streaking cultures on TSA plates supplemented with the tolerated maximum concentration of the sanitizer. Biofilms formed by the adaptive cells were determined by crystal violet staining and confocal laser scanning microscopy, and cell viability in the biofilms was examined by direct plating.

Results: The MIC of BZK on abovementioned strains was 64, 256, and 16 µg/mL, respectively. After adaptation, the maximum concentration of BZK at which the three strains could tolerate was 162, 192, and 154 µg/mL, respectively. Adaptive tolerance of the three strains to BZK was stable. The result shows previous adaptation to BZK enhanced ($P < 0.05$) biofilm formation of *S*. Typhimurium ATCC 14028, but not significantly ($P > 0.05$) affected the biofilm formation of the other two *Salmonella* strains. For the cell counts in the biofilm matrix, no substantial ($P > 0.05$) change was observed.

Significance: The data suggest that *Salmonella* has adaptability to BZK, and repeated use of BZK may pose a potential risk.

P3-260 Characterization of the Bacterial Community in a Floor Drain located in the Slaughtering Department of a Commercial Meat Processing Plant

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Developing Scientist Entrant

Introduction: Sanitation protocols are daily applied in food processing environments to control various spoilage and pathogenic microorganisms that can negatively affect product shelf life and food safety. Because floor drains (FD) are generally good representative of the processing area they are in, they can serve as a valuable hygiene indicator.

Purpose: The objective of this study was to characterize the bacterial community in a FD of the slaughtering department of a commercial meat processing plant over a one-year period.

Methods: Four times during a one-year period, the drain inner surface (IS), as well as the back of the covering grid (CG), were swabbed using sterile sponges on two occasions with a one-week interval. Swabs were taken prior to the passage of the animals. Samples were analyzed for total aerobic mesophilic (TAM; PCA, 35°C, 48 h) and *Enterobacteriaceae* (EB) counts using 3M Petrifilm™ (37°C, 24 h). After DNA extraction (QIAamp Power Fecal), sequencing of the 16S rRNA gene amplicons (V3-V4 region, long oligo-PCR approach, Illumina MiSeq) was used to characterize the bacterial population of the drain under study.

Results: For IS, enumeration did not vary significantly over the one-year period ($P>0.05$). However, significant differences were observed between the two samples taken at one-week interval $P<0.05$). No difference was fond for CG counts for the two samples taken at one-week interval ($P>0.05$). Alpha diversity (Shannon, $P<0,04$) and b-diversity (Wunifact, $P<0,055$) results suggest that the bacterial population changed when the drain is swabbed again a week later which reveals a certain degree of variation between samplings. Genera relative abundance indicated that although cell counts were similar for IS, bacterial population varied sporadically over a one-year period.

Significance:
The results suggest that the bacterial community within the drain sampled varied to a certain degree and that many factors, yet to be studied, influenced its composition.

P3-261 Validation of Small Interfering RNA to Knock-Down IRF3 Gene Related to Anti-Viral Factor in HepG2 Cells

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Introduction: Hepatitis A virus (HAV) is detected in various foods, and it causes hepatitis A. To detect HAV with a molecular technique, it should be cultured in mammalian cells, but the anti-viral factors of the cells decrease the efficiency of the culture. Thus, it is necessary to validate that the mammalian cells have knock-down anti-viral genes.

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Purpose: The objective of this study was to validate small interfering RNA (siRNA) to knock-down interferon regulatory factor 3 (IRF3), which increases the susceptibility of HepG2 cells, a human liver cancer cell, to HAV.

Methods: The siRNA (NM_001197122.1) was selected and used to verify the down-regulation effect of HepG2 cells. The siRNA was transfected to HepG2 cells, and HAV was infected on the siRNA-transfected HepG2 cells at 10^5 PFU/mL. After 3 h of culture at 37°C and 5% CO_2, the expression levels of the target gene IRF3 were evaluated by quantitative real-time PCR (qRT-PCR). The levels of cytokines (CXCL10 and CCL4) encoded by IRF3 in the siRNA-transfected HepG2 cells were evaluated by qRT-PCR.

Results: The siRNA knock-down the target gene of IRF3 in HepG2 cells, and the expression levels of the gene were significantly decreased (p<0.05) in the siRNA-transfected HepG2 cells, compared to non-transfected HepG2 cells. The cytokine levels of CXCL10 and CCL4 were subsequently reduced in the transfected HepG2 cells, compared to the negative control.

Significance: These results suggests that the knock-down of IRF3, using siRNA, increases the susceptibility of HepG2 cells to HAV infection.

P3-262 Identification of IRF7 Gene Role in Production of Anti-Viral Cytokines in HepG2 Cells by Knock-Down with Small Interfering RNA

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Introduction: Hepatitis A virus (HAV) causes foodborne diseases through raw food consumption. To detect HAV with a molecular technique, the amount of HAV needs to be increased sufficiently in mammalian cells, but mammalian cells have anti-viral factors, which decrease the incubation efficiency.

Purpose: The objective of this study was the development of small interfering RNA (siRNA) to knock-down interferon regulatory factor 7 (IRF7) related to the anti-viral activity of HepG2 cells to HAV.

Methods: The siRNA (NM_001572.3) that was verified for the down-regulation effect in the homo sapiens cell line was examined in HepG2 cells. The siRNA was transfected to the HepG2 cells and the HAV was infected on the siRNA-transfected HepG2 cells at 10^5 PFU/mL After the incubation at 37°C and 5% CO_2 for 3 h, the expression level of the target gene IRF7 was evaluated, and the anti-viral cytokines (CXCL10 and CCL4) effected by IRF7 were detected in the transfected HepG2 cells by quantitative real-time PCR.

Results: The siRNA sequences knock-down the target gene IRF7 in HepG2 cells successfully, and the expression levels of the gene were significantly decreased (p<0.05) in the transfected HepG2 cells, compared to the non-transfected HepG2 cells. The cytokine levels of CXCL10 and CCL4, compared to the negative control, were also reduced in the transfected cells.

Significance: These results suggests that siRNA can be used to knock-down IRF7, and the knock-down of IRF7 decreased the production of anti-viral cytokines, which may improve HAV incubation in HepG2 cells

P3-263 Quantitative Proteomic Analysis on the Slightly Acidic Eelectrolysed Water Triggered Viable but Non-Culturable (VBNC) *Listeria monocytogenes*

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Introduction: Slightly acidic electrolysed water (SAEW) is a sanitizer widely used in food industry. If sanitization is incomplete could induce *Listeria monocytogenes* to enter viable but non-culturable (VBNC) state.

Purpose: To identify the insufficient concentrations of SAEW treatment and hereby *L. monocytogenes* enter VBNC state. Then the VBNC bacteria were further validated and subjected to quantitative proteomic analysis for elucidation of VBNC *L. monocytogenes* formation mechanism.

Methods: We used low concentration of SAEW the VBNC state of *L. monocytogenes*, and used plating method in combination with flow cytometry to detect the VBNC bacteria and observed the morphology with scanning electron microscopy (SEM). The global proteomic profiles of the VBNC bacteria were established by tandem mass tag (TMT) labelled-LC-MS/MS.

Results: The chlorine concentration of SAEW to induce VBNC state was 8-10 mg/L and bacteria revealed the shrinkage of the cell membrane under SEM observation. The proteomic results indicated 203 differential expressed proteins (DEPs), including 78 up-regulated and 125 down-regulated DEPs. After Gene Ontology (GO) with Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, the significant DEPs were related to ribosome, biosynthesis of secondary metabolites and aminoacyl-tRNA biosynthesis. This study further identified 31 chlorinated peptides in the 22 chlorinated proteins to validate that SAEW induced the protein chlorination. The most prominent chlorinated proteins were identified as the elongation factor Tu and chaperone proteins. The 6 peptides were recognized as the RxxxxxcY motif.

Significance: To provide better understanding and formation mechanism of SAEW-induced VBNC bacteria.

P3-264 Comparative Genomic Analysis of *Vibrio parahaemolyticus* Isolated from Oysters, Seawater, and Clinical Samples

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Introduction: Vibrio parahaemolyticus is a foodborne pathogen that is commonly found in marine environments and seafood. The presence of different genes in *V. parahaemolyticus* contributes to its different ability in causing illness and resisting environmental stresses.

Purpose: This study aims to characterize and compare the genomes of *V. parahaemolyticus* isolated from oysters, seawater, and human clinical samples.

Methods: Fifty isolates of *V. parahaemolyticus* isolated from oysters, seawater, and infected humans in the U.S. were retrieved from the National Center for Biotechnology Information (NCBI) Genome Assembly Database. Annotation was performed on the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) web server. Pangenome matrices were built on Roary (v3.12.0). Statistical analyses and data visualization, including pangenome analysis, phylogenetic analysis, and heatmap, were performed on R (v4.2.2).

Results: Pangenome analysis revealed that *V. parahaemolyticus* isolated from oysters contained 11,312 genes. And compared with strains isolated from oysters and seawater, strains isolated from clinical samples contain the highest percentage of *tdh* and/or *trh* genes, which are recognized as the genes coding for the major virulence factor. In addition, genes associated with type III secretion system were more prevalent in clinical samples than in samples from oysters and seawater. Differences in the presence of genes involved in stress response, metabolism, and antibiotic resistance were observed among oysters, seawater, and clinical isolates.

Significance: This study helps to understand the genome-wide characteristics of *V. parahaemolyticus* isolated from oysters, seawater, and patients. It also aids in re-evaluating the food safety threats associated with *V. parahaemolyticus* and revealing the mechanism of survival, stress response, and virulence of *V. parahaemolyticus*.

Poster

P3-265 Rapid PCR-Lateral Flow Assay for the Onsite Detection of Atlantic White Shrimp

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❖ ***Developing Scientist Entrant***

Introduction: The Atlantic white shrimp (*Litopenaeus setiferus*) is of great economic importance to the United States and risks being substituted with inexpensive imported species such as *L. vannamei*.

Purpose: The aim of this study was to design and validate a robust multiplex PCR-lateral flow assay for the onsite identification of *L. setiferus* using a miniaturized, low-cost PCR instrument (WATSON® PCR).

Methods: For assay standardization, crude DNA was extracted from about 0.1 mm of tissue from 29 barcoded shrimp samples spread across five species (*L. setiferus, L. vannamei, P. muelleri, F. duorarum,* and *P. monodon*). White shrimp-specific primers and internal amplification control (IAC) were designed and labeled with FAM and biotin or FAM and digoxygenin, respectively. These modified primers were used in a multiplex PCR reaction to amplify the species-specific CO1 gene of *L. setiferus* and a conserved cytochrome oxidase 1 gene of other shrimp species. The two amplicons generated in the multiplex reaction were visually detected using the HybriDetect 2T lateral flow kit. The standardized assay was validated using 31 shrimp samples obtained from grocery stores. Cross-amplification potential of the assay was further validated with eight barcoded finfish samples. DNA yield and extraction time using different kits were compared using two-way ANOVA (SPSS software version 27) and GraphPad Prism version 9.4 was used to plot the graph.

Results: All *L. setiferus* samples simultaneously amplified both targets and generated three bands on the lateral flow test strips, whereas non-target species generated one or two bands in about 2 hours. The standardized assay showed 100% inclusivity for target *L. setiferus* samples and 100% exclusivity for non-target samples.

Significance: The assay standardized in this study can be used for onsite testing of *L. setiferus* samples at processing facilities, restaurants, and wholesalers' facilities and can be a robust tool for mitigating food fraud.

P3-266 Evaluation of Culture Methods to Detect *E. coli* in Raw Frozen Shell-on Shrimp and Raw Frozen Fish Fillet

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Introduction: Detection of *E. coli* in seafood falls under the Chilled or Frozen Foods section in Chapter 4 FDA-Bacteriological Analytic Manual (BAM). The reference procedure for frozen seafoods is the three tube (MPN) method. While seafood is not regulated for *E. coli* in US, other countries list control m and M values for shell on shrimp and fish fillet using film method AOAC 998.08.

Purpose: This work was done to evaluate some ready-to-use and solid phase agar methods for detection of *E. coli* in comparison to the reference method.

Methods: Frozen shell on shrimp and fish fillet were purchased. *E. coli* (ATCC 11775 and 25922) cultured in TSB broth were spiked , refrozen, and thawed for analysis. Five replicates of three test levels were tested in duplicate. Methods evaluated were Violet Red Bile Agar (VRBA) or Modified (TSA-bottom and VRBA-overlay) pick confirmed EC-MUG (EMD) 35±1°C 24±2 hours, AOAC 998.08 Petrifilm EC 35±1°C 24±2 hours (3M/Neogen), three tube MPN using LST-MUG(EMD) 35±1°C 48±3 hours, Peel Plate EC (PP-EC) (Charm Sciences Inc.) 35±1°C 24±2 hours. Statistical paired and unpaired analysis N=10 v.1.2 LaBudde.

Results: Recovery of *E. coli* in both matrices were in this highest to lowest order: Mod-VRBA or VRBA> PP-EC>MPN>AOAC 998.08. Upper 95% confidence interval (UCL) greater than 0.5 log and lower 95% confidence interval (LCL) less than -0.5 log to the methods log differences were used to determine significant differences in method results. In fish fillet all methods were equivalent. In shrimp shells MPN, AOAC 998.08 and PP-EC were equivalent in unpaired analysis; whereas, method PP-EC was significantly greater in paired analysis from AOAC 998.08 <0.5 UCL in paired analysis. AOAC 998.08 LCL -0.8-1.2 and PP-EC LCL -0.5 were significantly lower than Mod-VRBA.

Significance: Methods to evaluate sanitation should be first verified with spiking the intended test matrix and comparing results to reference methods.

P3-267 Culture Dependent vs. Culture Independent 16S Sequencing for Bacterial Communities during Decomposition of Shrimp

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Introduction: Understanding bacterial community changes of seafood during storage is critical in developing methods for bacterial biomarkers of decomposition. However, culture dependent and independent methods may yield different results when evaluating bacterial community compositions.

Purpose: The study objective was to compare culture dependent and independent 16S sequencing methods to evaluate bacterial community composition during storage of shrimp at different temperatures.

Methods: Expired beheaded shrimp were incubated at 0, 12, 24, and 36ºC for 20 days, 72, 24, and 12 hours, respectively. At each sampling point, triplicate samples were collected and metagenomic analysis using the16S rRNA gene amplicons with 26F1 and 534R1 primers was conducted (culture independent). Samples were homogenized (1:10), spread plated on TSA, and incubated under the same conditions as indicated above. DNA from 48 colonies at each sampling point was purified for 16S rRNA gene sequencing via Sanger Sequencing using 27F and 1492R primers (culture dependent).

Results: At 0°C, culture dependent results showed the initial bacterial community consisted primarily of *Psychrobacter*, *Arcobacter*, and *Planococcus* spp. By day 20, it consisted predominantly of *Shewanella* spp. The culture independent method detected *Shewanella* spp. initially and tracked its increase throughout storage. The presence/increase of *Shewanella* spp. would be observed on different days based on the two methods. At 36°C, based on both methods, the initial bacterial community was highly diverse. However, the culture dependent method showed the dominant bacteria were *Vibrio* and *Photobacterium* spp. by hour 12, whereas the culture independent method demonstrated a much less pronounced decrease in diversity. Generally, the culture independent method identified different species, compared to culture dependent, and showed a more diverse community composition.

Significance: Understanding the difference in composition and changes of bacterial communities during decomposition at various temperatures and assessing the most effective and reliable methods for evaluation will aid in the detection of seafood decomposition and may ultimately help identify bacterial biomarkers thereof.

P3-268 Antibacterial Activity of the Sea Cucumber *Holothuria leucospilota* Whole-Body Extract Against Methicillin-Resistant and Enterotoxin-Producing *Staphylococcus aureus* Strains

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Introduction: Preformed enterotoxins of *Staphylococcus aureus* are one of the most common causes of seafood-borne food poisoning worldwide. Aquatic organisms, including those used as seafood, are also a source of organic compounds of potential medical importance.

Purpose: Within the framework of an antimicrobial activity study of marine macro-organisms from the Persian Gulf, bioactive compounds of the sea cucumber *Holothuria leucospilota* were extracted from whole sea cucumber bodies using chloroform or methanol. The extracts were evaluated for their antibacterial effects against methicillin-resistant *Staphylococcus aureus* (MRSA) and enterotoxin producing *Staphylococcus aureus strains* (SEASA, SEBSA).

Methods: Antimicrobial activities were determined using three methods: disk diffusion tests, minimum bactericidal concentration (MBC), and minimum inhibitory concentration (MIC)

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Results: The results demonstrate that methanol and chloroform extracts have an inhibitory effect on the growth of all strains tested at MIC concentrations up to 100 mg/ml. Also, the chloroform extract demonstrated bactericidal activity against SEBSA in concentrations of about 100 mg/ml. The extract below 100 mg/ml concentrations also shows bactericidal effects against MRSA and SEBSA. The highest antibacterial activity was found in the methanol extract.

Significance: Therefore, sea cucumber extract is an interesting candidate for the identification of new antimicrobials, however, comprehensive investigations are needed to separate and identify the active compounds from *Holothuria leucospilota* from the Persian Gulf.

Key words: sea foods, Bacteria, Sea cucumber, *Staphylococcus aureue,* Antibiotic

P3-269 A Study on the Prevalence of Toxin Genes Antimacrobial Suseptibility of *Staphylococcus Aureuus* Isolates in Marine and Farmed Fish in Iran

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Introduction: Globally, *Staphylococcus aureus* is a leading cause of seafood-borne disease, which is due to the contamination of food by preformed enterotoxins.

Purpose: This study investigated the contamination of fishery products before they were purchased and consumed.

Methods: In this study, a total of 123 (40.7%) *Staphylococcus aureus* strains were obtained from 300 fish fresh and frozen marine and aquaculture samples and were tested by PCR for their antimicrobial susceptibility. We assessed the prevalence of the genes responsible for the staphylococcal enterotoxins (SEA, SEB) and toxic shock syndrome toxin 1 (TSST-1) genes.

Results: The results indicated that 40.7% of aqua food samples were contaminated with *S. aureus*, and16.8% of these isolates were mec-A-positive. Sixty-two percent of the strains isolated from contaminated seafood was enterotoxigenic *S. aureus*, and21.2% of SEs were MRSA-positive. The most prevalent genotype was characterized by the presence of the sea gene (35.2%), followed by the seb gene (11.5%), and the tst gene encoding TSST-1 was found in eight strains (2.7 %). Of the 103 *S. aureus* isolates.243strains (81.1 %) were resistant to at least one antibiotic.

Significance: To prevent the contamination of fishery products in Iran, mandatory programmes should be revised to facilitate improved hygiene practices during fishing, aquaculture, processing, and sales to prevent the outbreaks of enterotoxigenic MRSA.

P3-270 Effects of High Pressure Processing on the Microbial and Chemical Qualities, and Bacterial Microbiota of Freshwater Clam during Cold Storage

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Introduction: The high-pressure processing (HPP) has been primarily applied for the treatment of saltwater bivalve such as oysters, scallops, and mussels, but research on the effects of HPP on shucking, pasteurization, and freshness preservation of the freshwater bivalve is scarce.

Purpose: This aim of study was to evaluate the impacts of HPP (200- 600 MPa for 3 min) on the microbial, chemical, and organoleptic properties and the bacterial flora of freshwater clam (*Corbicula fluminea*) during cold storage.

Methods: The microbe deactivation, delay of the loss of chemical and organoleptic qualities, and prolongation of shelf life of the freshwater after HPP treatment were determined. In addition, using high throughput sequencing (HTS) technology, we also investigated the differences in microflora between untreated clam meat samples and samples subjected to HPP at 300 and 500 MPa.

Results: The results indicated that with an increase in pressure, the expanding rate, moisture content, shucking ratio, pH, lightness (L^*), and whiteness (W) increased, whereas redness (a^*), yellowness (b^*), aerobic plate count (APC), psychrotrophic bacteria count (PBC), coliform, and *Salmonella* spp. decreased. In addition, HPP treatment of >300 MPa on samples significantly retarded the increases in APC, PBC, coliform, *Salmonella* spp., and total volatile basic nitrogen and a drop in pH during cold storage. Results of organoleptic analysis showed that HPP at 300 and 500 MPa prolonged the shelf life from 6 days (control group) to 9 days and 12 days, respectively. High-throughput sequencing (HTS) revealed that HPP considerably changed the spoilage microflora in samples after 15 days storage and delayed the spoilage process.

Significance: These results demonstrate that pressurization at least 300 MPa for 3 min on clam samples were able to reach 100% shucking, inactivate microbial growth, and retard quality loss, while they effectively extended shelf life of freshwater clam during cold storage.

P3-271 Structural Characterization and Gel Properties of *Porphyra yezoensis* Polysaccharide: A New Potential Source of Hydrocolloids

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Introduction: *Porphyra yezoensis* is one of the most economically valuable marine algae in China, containing polysaccharides, proteins, peptides and other nutrients, with polysaccharides being its predominant component. *Porphyra yezoensis* polysaccharide (PSPY) studies currently focus on extraction optimization, structural modification, and biological activity. Yet, little is known about the potential of PSPY as hydrocolloids in food applications.

Purpose: Determining the chemical structure and gelation mechanism of PSPY establishes the basis for promoting the development of PSPY as a sustainable food resource.

Methods: PSPY was extracted by ultrasonic-assisted alcohol precipitation, and the structure of PSPY was identified by high-performance liquid chromatography, high-performance gel permeation chromatography and nuclear magnetic resonance spectroscopy. Ca^{2+} solutions (0, 3, 6, 9 mM) were mixed with PSPY solutions to investigate the effects of Ca^{2+} on the rheological properties, textural analysis and structure of PSPY gels.

Results: The molecular weight of PSPY was 17.16 kDa, and the main chain is (1→3)G4Sβ(1→3)G(1→6)G4Sα(1→4)LA(1→6)G4Sα. The rheological data indicated that the addition of Ca^{2+} (≤ 6 mM) was beneficial to increase the apparent viscosity of Ca^{2+}-PSPY gels. Textural properties, scanning electron microscopy and fractal dimension reveal that the addition of Ca^{2+} improves the network structure of Ca^{2+}-PSPY gels. Fourier transform infrared spectroscopy, X-ray diffraction and thermogravimetric studies confirmed that Ca^{2+} altered polymer intermolecular interactions. In addition, Ca^{2+} can also form stable calcium bridges with the carboxyl groups in PSPY, which plays an important role in promoting gel formation and maintaining the gel network structure.

Significance: This work reveals the unique structure and gelling behavior of PSPY and promotes the application of PSPY as a novel hydrocolloid in the food industry.

Poster

P3-272 Characterization of the Resistome and Virulome on Antimicrobial-Resistant *E. coli* isolated from Meat, Vegetables, and Surface Water Samples

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❖ ***Developing Scientist Entrant***

Introduction: The antimicrobial resistance crisis has highlighted the importance of the environment and food as reservoirs of resistant bacteria. Then, contamination of raw food and water sources with antimicrobial-resistant *E. coli* has become an emerging concern in food safety.

Purpose: To characterize multidrug-resistant (MDR) *E. coli* obtained from different foods and water sources.

Methods: Samples from vegetables (n=480), raw meat (n=113), and surface water (n=32) were collected from Molina, a city in central Chile, over a year. Samples were plated in MacConkey Agar supplemented with ciprofloxacin (CIP) and ceftazidime (CAZ) to isolate resistant *E. coli*. The disk diffusion method was used to characterize the isolate's antimicrobial susceptibility, and Multidrug resistance (MDR) isolates were sequenced. The resistome, virulome, phylogroup and MLST profile of the MDR *E. coli* were determined and a phylogenetic analysis based on SNPs was performed, using the bioinformatics tools abrictae, virulencefinder, ezclermont, mlst and RaxML respectively.

Results: A total of 295 *E. coli* isolates were obtained of wich 73,9% (68/92) of vegetables isolates, 82,7% (24/29) of water isolates and 39,1% (68/174) of meat isolates presented a MDR profile. Genes aph(3")-Ib gene (60/160), beta-lactamases blaTEM (84/160), and blaCTX-M (53/160) were found the most frequently identified. Also, the mcr-1 gene, which confers resistance to colistin and is globally disseminated, was identified in two isolates from vegetables. Furthermore, 12 virulence genes (*AslA, anr, csgA, fdeC, fimH, hlyE, nlpI, terC, yehA, yehB, yehC* and *yehD*) were found in more than 94% of the analyzed genomes. The most frequently found phylogroup was A (41/160), and sequence type ST131 was the most prevalent (26/160).

Significance: We isolated relevant MDR *E. coli* containing virulence determinants in raw foods and water sources. Our results highlight the need for food safety control and surveillance systems to minimize the dissemination of MDR *E. coli.*

P3-273 Genomic Analysis Reveals Long-Term *Salmonella* spp. Persistence in Surface Waters in Chile

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Introduction: Salmonella has been detected in surface waters in Chile; these waters are a primary source of irrigation for produce and could reach the consumer, so *Salmonella* characterization will help to identify public health hazards.

Purpose: To Investigate the diversity and persistence of *Salmonella* spp. in surface waters through genomic analysis.

Methods: Salmonella isolates were collected from four watersheds in two Chilean regions (Metropolitan and Maule) between May 2019 and July 2022. Over 1,200 isolates were sequenced at FDA/CFSAN on Illumina platforms. FDA/CFSAN pipeline was used for quality control and genome assembly. Ridom Seqspehere+ was used to determine the Sequence Types (ST) of 592 genomes and to investigate phylogenetic relatedness among genomes. Serotypes were predicted with SISTR.

Results: We identified 69 different ST; ST32 (69/592; 11.7%) was the most common, followed by ST19 (54/592; 9.1%) and ST11 (43/592; 7.1%). The most frequently found serotypes were *S.* Typhimurium (71/592; 12.0%), Infantis (69/592; 11.7%) and Newport (48/592; 8.1%). Within serotypes, cgMLST allele differences ranged from 2,737 (Newport) to 2,829 (Enteritidis). Genomes with indistinguishable cgMLST profiles were detected among isolates obtained in the same region but in different watersheds (e.g., Newport in Maipo and Mapocho watersheds) and in different regions (e.g., Enteritidis in Metropolitan and Maule Region). We also found indistinguishable genomes isolated on the same date but across different locations in a watershed (e.g., Infantis, Newport, Agona) and from different dates in the same sampling site (e.g., Typhimurium, Enteritidis). Moreover, highly related genomes (<5 cgMLST allele differences) were widely distributed across dates, locations, watersheds, and regions.

Significance: Some *Salmonella* clones persist for months in surface waters in Chile, while highly related isolates are widely spread in watersheds from two regions in Chile. Persistent clones need to be fully characterized to assess the risk they might pose to food production and public health.

P3-274 Comparative Genomic Analyses of *Salmonella* Typhimurium, Newport, and Infantis Isolates from Surface Waters in Latin America, 2019–2022

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Introduction: Surface waters are considered important ecological niches where *Salmonella enterica* can persist and disseminate to food production environments.

Purpose: The purpose of this study was to apply whole-genome sequencing to characterize *S.* Typhimurium (ST), Newport (SN), and Infantis (SI) isolates from surface waters in Chile, Mexico, and Brazil during 2019-2022.

Methods: ST (n = 106), SN (n = 162), and SI (n = 113) isolates from surface waters in Chile, Mexico, and Brazil were sequenced using MiSeq or NextSeq. Based on the whole-genomic data, antimicrobial resistance genes (ARGs), plasmids, and sequence type of each isolate were identified using ResFinder, PlasmidFinder, and mlst. Single nucleotide polymorphisms (SNPs) were called and whole-genome phylogeny was then inferred for each serotype using CFSAN SNP Pipeline.

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Results: ARGs were present in 17.7%, 64.3%, and 12.5% of ST isolates, 2.0%, 35.6%, and 2.6% of SN isolates, and 72.6%, 50.0%, and 5.6% of SI isolates from Chile, Mexico, and Brazil, respectively. Plasmids were identified in 96.8%, 89.3%, and 100.0% of ST isolates, 7.8%, 41.1%, and 2.6% of SN isolates, and 76.7%, 36.4%, and 5.6% of SI isolates from Chile, Mexico, and Brazil, respectively. Among those carrying ARGs, ST and SN isolates from all countries contained plasmids, while plasmids were detected in 100.0%, 72.7%, and 0.0% of SI isolates from Chile, Mexico, and Brazil, respectively. The most common sequence types were 19, 213, and 19 for ST isolates, and 31, 132, and 164 for SN isolates from Chile, Mexico, and Brazil, respectively, whereas the most common sequence type was 32 for SI isolates from all countries. Isolates for each serotype clustered by country, with those from different countries genetically distant (SNPs>20).

Significance: This study highlights the genetic diversity among ST, SN, and SI isolates from surface waters in Latin America, which provides critical genomics-informed data of public health significance for pathogen surveillance.

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